

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C12N 15/31, A61K 39/02, 39/106</b>		A1	(11) International Publication Number: <b>WO 97/20050</b>  (43) International Publication Date: <b>5 June 1997 (05.06.97)</b>						
<p>(21) International Application Number: <b>PCT/AU96/00767</b></p> <p>(22) International Filing Date: <b>29 November 1996 (29.11.96)</b></p> <p>(30) Priority Data:</p> <table><tr><td>PN 6910</td><td>30 November 1995 (30.11.95)</td><td>AU</td></tr><tr><td>PN 6911</td><td>30 November 1995 (30.11.95)</td><td>AU</td></tr></table> <p>(71) Applicants (<i>for all designated States except US</i>): DARATECH PTY. LTD. [AU/AU]; 3rd floor, 493 St. Kilda Road, Melbourne, VIC 3004 (AU). PIG RESEARCH AND DEVELOPMENT CORPORATION [AU/AU]; Industry House, 3rd floor, Brisbane Avenue, Barton, ACT 2600 (AU).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (<i>for US only</i>): PANACCIO, Michael [AU/AU]; 122 Hill Road, North Balwyn, VIC 3104 (AU). HASSE, Detlef [AU/AU]; 4 Scullin Court, Sunbury, VIC 3429 (AU).</p> <p>(74) Agents: HUGHES, E., John, L. et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, VIC 3000 (AU).</p>		PN 6910	30 November 1995 (30.11.95)	AU	PN 6911	30 November 1995 (30.11.95)	AU	<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i></p>	
PN 6910	30 November 1995 (30.11.95)	AU							
PN 6911	30 November 1995 (30.11.95)	AU							
<p>(54) Title: <b>THERAPEUTIC AND DIAGNOSTIC COMPOSITIONS</b></p> <p>(57) Abstract</p> <p>The present invention relates generally to therapeutic compositions for the treatment and/or prophylaxis of intestinal disease conditions in animals and birds caused or exacerbated by <i>Lawsonia intracellularis</i> or similar or otherwise related microorganism. The present invention also contemplates methods for the treatment and/or prophylaxis of such intestinal disease conditions and to diagnostic agents and procedures for detecting <i>Lawsonia intracellularis</i> or similar or otherwise related microorganism.</p>									

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LJ	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

## THERAPEUTIC AND DIAGNOSTIC COMPOSITIONS

The present invention relates generally to therapeutic compositions for the treatment and/or prophylaxis of intestinal disease conditions in animals and birds caused or exacerbated by *Lawsonia intracellularis* or similar or otherwise related microorganism. The present invention also contemplates methods for the treatment and/or prophylaxis of such intestinal disease conditions and to diagnostic agents and procedures for detecting *Lawsonia intracellularis* or similar or otherwise related microorganism.

10

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

15

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

20

The meat industry in Australia and, indeed, in most countries of the world, is an important aspect of the overall livestock industry. However, the meat industry is subject to rapid economic downturn in response to disease conditions affecting the animals as well as human diseases putatively carried by the animals. It is important, therefore, to have well defined 25 treatment, prophylactic and diagnostic procedures available to deal with infections or potential infections in animals and humans.

Pigs form a major component of the meat industry. However, pigs are sensitive to a wide spectrum of intestinal diseases collectively referred to as porcine proliferative enteropathy 30 (PPE). This disease has previously been known as intestinal adenomatosis complex (1),

- 2 -

porcine intestinal adenomatosis (PIA), necrotic enteritis (2), proliferative haemorrhagic enteropathy (3), regional ileitis (4), haemorrhagic bowel syndrome (5), porcine proliferative enteritis and *Campylobacter* spp induced enteritis (6).

5 There are two main forms of PPE: a non-haemorrhagic form represented by intestinal adenomatosis which frequently causes growth retardation and mild diarrhoea; and a haemorrhagic form, which is often fatal, represented by proliferative haemorrhagic enteropathy (PHE) where the distal small intestine lumen becomes engorged with blood. PPE has been reported in a number of animal species including pigs (14), hamsters (7), ferrets (15), guinea 10 pigs (16), rabbits (17) as well as avian species (18).

The causative organism of PPE is a *Campylobacter*-like organism referred to herein as "Lawsonia intracellularis" (26). The organism has also been previously referred to as *Ileal symbiont intracellularis* (7). PPE-like diseases in pigs may also be caused by other pathogens 15 such as various species of *Campylobacter* (8).

*Lawsonia intracellularis* is an intracellular, possibly obligate intracellular, bacterium. It can only be cultured *in vitro* with tissue culture cells (9, 26). Pigs suffering from PPE are characterised by multiple abnormal immature crypts and *L. intracellularis* is located in the 20 cytoplasm of these crypt cells.

PPE is a significant cost component associated with the pig industry, especially in terms of stock losses, medication costs, reduced growth rates of pigs and increased feed costs. PPE also contributes to downstream indirect costs in, for example, additional labour costs and 25 environmental costs in dealing with antibiotic residue contamination and in control measures to prevent the organism being passed on or carried to other animals or humans.

Current control strategies for PPE rely on the use of antibiotics. However, such a strategy is considered to be short to medium term especially as governmental regulatory pressures tend to 30 target animal husbandry practices which are only supported by prophylactic antibiotics. There

- 3 -

is a need, therefore, to develop effective, safe and low cost alternatives to the use of antibiotics. There is also a need to extend this alternative to antibiotics to similar organisms which infect other animals such as humans.

- 5 In work leading up to the present invention, the inventors sought to develop vaccines for the prophylaxis and treatment of PPE in animals and birds. The vaccines of the present invention provide an efficacious alternative to the use of antibiotics with a range of consequential husbandry and medical benefits.
- 10 Accordingly, one aspect of the present invention provides a vaccine composition for the prophylaxis or treatment of infection in an animal or bird by *L. intracellularis* or similar or otherwise related microorganism, said vaccine composition comprising an immunogenic, non-pathogenic form of *L. intracellularis* or related microorganism or an immunogenic component thereof and one or more carriers, diluents and/or adjuvants suitable for veterinary or  
15 pharmaceutical use.

The present invention is particularly useful and is exemplified hereinafter in relation to the protection and/or treatment of pigs from infection with *L. intracellularis*. However, this is done with the understanding that the present invention extends to the prophylaxis and treatment of  
20 all animals including humans and birds from infection with *L. intracellularis* and/or related microorganisms. Animals contemplated by the present invention include but are not limited to humans, primates, companion animals (e.g. cats, dogs), livestock animals (e.g. pigs, sheep, cattle, horses, donkeys, goats), laboratory test animals (e.g. mice, rats, guinea pigs, rabbits) and captive wild animals (e.g. kangaroos, foxes, deer). The present invention also extends to birds  
25 such as poultry birds, game birds and caged birds.

Furthermore, the present invention extends to all isolates and sub-types of *L. intracellularis* as well as other species of the genus *Lawsonia* or other microorganisms related thereto at the nucleotide, biochemical, structural, physiological and/or immunointeractive level. Reference  
30 hereinafter to "*Lawsonia intracellularis*" or its abbreviation "*L. intracellularis*" includes all

microorganisms similar to or otherwise related to this microorganism. For example, a related microorganism may have a nucleotide sequence similarity at the chromosome or extrachromosomal level of at least about 60%, more preferably at least about 70% and even more preferably greater than at least about 80% with respect to all or part of a nucleotide sequence within the chromosome or extrachromosomal elements of *L. intracellularis*. For example, these percentage similarities may relate to the sequence set forth in SEQ ID NO:5. This sequence is a portion of the *L. intracellularis* chromosome.

Accordingly, this aspect of the present invention is directed to a vaccine composition for the prophylaxis and/or treatment of infection in a pig by *L. intracellularis*, said vaccine composition comprising an immunogenic, non-pathogenic form of *L. intracellularis* or related microorganism or an immunogenic component thereof and one or more carriers, diluents and/or adjuvants suitable for veterinary or pharmaceutical use.

15 The term "immunogenic component" refers to *L. intracellularis* (in attenuated non-pathogenic or killed form) or a component of *L. intracellularis* including a peptide, polypeptide or a protein encoded by DNA from or derived from *L. intracellularis* which is capable of inducing a protective immune response in a pig. A protective immune response may be at the humoural and/or cellular level and generally results in a substantial reduction in the symptoms of PPE in 20 pigs. The vaccine compositions will comprise an effective amount of immunogenic component such as to permit induction of a protective immune response.

According to this aspect of the present invention there is provided a vaccine composition for the prophylaxis and treatment of a pig by *L. intracellularis*, said vaccine composition comprising an amount of at least one immunogenic component from *L. intracellularis* or related microorganism effective to induce a protective immune response in said pig against *L. intracellularis* or related microorganism, said vaccine composition further comprising one or more carriers, adjuvants and/or diluents suitable for veterinary or pharmaceutical use.

30 The immunogenic component may be a naturally occurring peptide, polypeptide or protein, a

- 5 -

carbohydrate, lipid or nucleic acid (e.g. DNA) or any combination thereof isolated from *L. intracellularis* or a cell culture thereof or a recombinant form of a peptide, polypeptide or protein encoded by DNA from or derived from *L. intracellularis* or is a derivative of said peptide, polypeptide or protein.

5

An isolated component of *L. intracellularis* is a component which has undergone at least one purification step or which has undergone at least partial concentration from a cell culture comprising *L. intracellularis* or from a lysed preparation of *L. intracellularis* cells. The purity of such a component from *L. intracellularis* which has the requisite immunogenic properties 10 is preferably at least about 40%, more preferably at least about 50%, even more preferably at least about 60%, still more preferably at least about 70% and even more preferably at least about 80-90% or greater relative to other components in a preparation as determined by molecular weight, immunogenic activity or other suitable means.

15 A particularly useful form of the vaccine is a whole cell vaccine which comprises *L. intracellularis* in attenuated or otherwise non-pathogenic form or killed cells or various fractions thereof.

Attenuated or non-pathogenic cells include killed *L. intracellularis* cells prepared, for example, 20 by heat, formalin or other chemical treatment, electric shock or pressure and such cells are particularly useful in the practice of the present invention.

According to this aspect of the present invention there is provided a vaccine composition for the prophylaxis and/or treatment of infection in a pig by *L. intracellularis* or related 25 microorganism said vaccine composition comprising a killed preparation of *L. intracellularis* or related microorganism or an immunogenic fraction thereof and one or more carriers, diluents and/or adjuvants suitable for veterinary or pharmaceutical use.

In an alternative embodiment, a recombinant vaccine may be employed. The recombinant 30 vaccine may comprise one or more recombinant peptides, polypeptides or proteins derived from

*L. intracellularis* or is a recombinant molecule immunologically related to a peptide, polypeptide or protein derived from *L. intracellularis* or may be a fusion molecule having a first portion comprising a peptide, polypeptide or protein derived from *L. intracellularis* and a second heterologous peptide, polypeptide or protein which may be useful, for example, as a 5 carrier molecule or an adjuvant or an immune stimulating molecule such as cytokine. A particularly useful recombinant protein from *L. intracellularis* comprises a peptide, polypeptide or protein derived from the cell surface or membrane of *L. intracellularis*, is an enzyme in a metabolic pathway within *L. intracellularis* or is a refolding and/or heatshock protein. In a preferred embodiment, the protein is a refolding/heatshock protein such as but not limited to 10 GroEL and GroES. Other putative vaccine candidates include flagellar basal body rod protein, S-adenosylmethionine: tRNA ribosyltransferase-isomerase, enoyl-(acyl-carrier-protein) reductase, N-acetyl muramoyl-L-alanine amidase (autolysin), UOP-3-O-[3-hydroxymyristoyl] glucosamine N-acetyltransferase and a glucarate transporter.

15 According to a preferred embodiment, the present invention relates to a vaccine composition for the prophylaxis and/or treatment of infection in a pig by *L. intracellularis* or related microorganism, said vaccine composition comprising at least one recombinant peptide, polypeptide or protein from *L. intracellularis* and wherein said recombinant peptide, polypeptide or protein is capable of inducing a protective immune response against *L. 20 intracellularis* in pigs, the vaccine composition further comprising one or more carriers, diluents and/or adjuvants suitable for veterinary or pharmaceutical use.

In a particularly preferred embodiment, the recombinant protein is GroEL having an amino acid sequence as set forth in SEQ ID NO:2 or is a protein having a predicted amino acid sequence 25 with at least about 40%, at least about 60%, or more preferably at least about 70% and even more preferably at least about 80-90% or greater similarity to all or part of the amino acid sequence set forth in SEQ ID NO:2.

In another embodiment, the recombinant molecule is GroES having an amino acid sequence as 30 set forth in SEQ ID NO:4 or is a molecule having an amino acid sequence at least about 40%,

- 7 -

at least about 60%, more preferably at least about 70% and even more preferably at least about 80-90% or greater similarity to all or part of the amino acid sequence set forth in SEQ ID NO:4.

Another embodiment of the present invention includes and comprises a peptide, polypeptide 5 or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:1 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

10 In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:3 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

15

In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:5 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related 20 microorganism.

In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:6 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and 25 which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:8 or having at least 30 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and

- 8 -

which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

In a related embodiment, the present invention includes and comprises a peptide, polypeptide 5 or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:11 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

10 In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:13 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

15

In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:15 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related 20 microorganism.

In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:17 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and 25 which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:18 or having at least 30 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and

which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

In a related embodiment, the present invention includes and comprises a peptide, polypeptide 5 or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:19 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

10 In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:20 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

15

In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:21 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related 20 microorganism.

In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:22 or having at least 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and 25 which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

In a related embodiment, the present invention includes and comprises a peptide, polypeptide or protein encoded by a nucleotide sequence as set forth in SEQ ID NO:23 or having at least 30 40% similarity thereto or capable of hybridizing thereto under low stringency conditions and

- 10 -

which nucleotide sequence encodes an immunogenic component of *L. intracellularis* or related microorganism.

Preferred percentage similarities include at least about 50% or at least about 60% or at least 5 about 70-90%.

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions.

10 Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and 15 from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.

The present invention also contemplates peptides, polypeptides or proteins having an amino acid sequence substantially as set forth in one of SEQ ID NO:7 or 9 or 10 or 12 or 14 or 16 or 20 having at least 40% similarity thereof or to all or part thereof. Preferred percentage similarities include at least about 50%, or at least about 60% or at least about 70-90%.

The present invention further extends to a vaccine comprising a recombinant vaccine vector encoding a peptide, polypeptide or protein derived from *L. intracellularis* or related 25 microorganism as described above. The vaccine vector may be of viral, yeast or bacterial origin and would be capable of expression of a genetic sequence encoding a peptide, polypeptide or protein from *L. intracellularis* in a manner effective to induce a protective immune response. For example, a non-pathogenic bacterium could be prepared containing a recombinant sequence capable of encoding a peptide, polypeptide or protein from *L. intracellularis*. The recombinant 30 sequence would be in the form of an expression vector under the control of a constitutive or

- 11 -

inducible promoter. The bacterium would then be permitted to colonise suitable locations in a pig's gut and would be permitted to grow and produce the recombinant peptide, polypeptide or protein in amount sufficient to induce a protective immune response against *L. intracellularis*.

5

In a further alternative embodiment, the vaccine may be a DNA vaccine comprising a DNA molecule encoding a peptide, polypeptide or protein from *L. intracellularis* and which is injected into muscular tissue or other suitable tissue in a pig under conditions sufficient to permit transient expression of said DNA to produce an amount of peptide, polypeptide or 10 protein effective to induce a protective immune response.

The vaccines of the present invention may contain a single peptide, polypeptide or protein or a range of peptides, polypeptides or proteins covering different or similar epitopes. In addition, or alternatively, a single polypeptide may be provided with multiple epitopes. The latter type 15 of vaccine is referred to as a polyvalent vaccine. A multiple epitope includes two or more repeating epitopes.

The formation of vaccines is generally known in the art and reference can conveniently be made to Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pennsylvania, 20 USA.

The present invention, therefore, contemplates a pharmaceutical composition or vaccine composition comprising an immunity developing effective amount of one or more of:

- 25 (i) an immunogenic component from *L. intracellularis*;
- (ii) a recombinant peptide, polypeptide or protein from *L. intracellularis* having immunogenic properties; and/or
- (iii) whole cells or a component or fraction thereof from *L. intracellularis*.

30 The above components are referred to hereinafter as "active ingredients". The active

- 12 -

ingredients of a vaccine composition as contemplated herein exhibit excellent therapeutic activity, for example, in the treatment and/or prophylaxis of PPE when administered in an amount which depends on the particular case. For example, for recombinant molecules, from about 0.5 µg to about 20 mg may be administered. Other useful effective amounts include 1  
5 µg to about 10 mg, 10 µg to about 5 mg and 50 µg to about 1 mg. The important feature is to administer sufficient to induce an effective protective immune response. The above amounts may be administered as stated or may be calculated per kilogram of body weight. Dosage regime may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated  
10 by the exigencies of the therapeutic situation. Booster administration may also be required.

The active ingredients may be administered in a convenient manner such as by the oral, intravenous (where water soluble), intramuscular, subcutaneous, intranasal, intradermal or suppository routes or implanting (eg using slow release technology). Depending on the route  
15 of administration, the active ingredients which comprise, for example, peptides, polypeptides or proteins may be required to be coated in a material to protect said ingredients from the action of enzymes, acids and other natural conditions which may inactivate said ingredients.

The term "adjuvant" is used in its broadest sense and includes any immune stimulating  
20 compound such as interferon. Adjuvants contemplated herein include resorcinols, non-ionic surfactants such as polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether and Freund's complete and incomplete adjuvant.

The active compounds may also be administered parenterally or intraperitoneally. Dispersions  
25 can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where  
30 water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile

injectable solutions or dispersion. In all cases the form must be fluid to the extent that easy syringability exists unless the pharmaceutical form is a solid or semi-solid such as when slow release technology is employed. In any event, it must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of 5 microorganisms.

The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for 10 example, by the use of a coating such as licithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. 15 Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as 20 required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a 25 powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

Carriers and diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media 30 and agents in vaccines is well known in the art. Except insofar as any conventional media or

- 14 -

agent is incompatible with an active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

- 5 Still another aspect of the present invention is directed to antibodies to the peptides, polypeptides or proteins from *L. intracellularis* or recombinant forms thereof or non-proteinaceous molecules such as carbohydrates. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to *L. intracellularis* or may be specifically raised to specific molecules or whole cells or components or fractions thereof.
- 10 The antibodies of the present invention are particularly useful for immunotherapy and vaccination and may also be used as a diagnostic tool for infection or for monitoring the progress of a vaccination or therapeutic regime.

For example, recombinant *L. intracellularis* peptides, polypeptides or proteins can be used to  
15 screen for naturally occurring antibodies to *L. intracellularis*. Alternatively, specific antibodies can be used to screen for *L. intracellularis*. Techniques for such assays are well known in the art and include, for example, sandwich assays and ELISA. Hereinafter, an immunogenic component is considered to encompass an immunogenic component of *L. intracellularis* and includes recombinant molecules, whole cells and cell extracts.

20 In accordance with this aspect of the present invention, the immunogenic components are particularly useful in screening for antibodies to *L. intracellularis* and, hence, provide a diagnostic protocol for detecting *L. intracellularis* infection. Alternatively, biological samples can be directly screened for *L. intracellularis* using antibodies raised to immunogenic  
25 components.

Accordingly, there is provided a method for the diagnosis of *L. intracellularis* infection in a pig comprising contacting a biological sample from said pig with an immunogenic component binding effective amount of an antibody for a time and under conditions sufficient for an  
30 immunogenic component-antibody complex to form, and then detecting said complex.

- 15 -

The presence of immunogenic components (or antibodies thereto) in a pig's blood, serum, or other bodily fluid, can be detected using a wide range of immunoassay techniques such as those described in US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. This includes both single-site and two-site, or "sandwich", assays of the non-competitive types, as well as in the traditional 5 competitive binding assays. Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention.

- 10 Briefly, in a typical forward assay, an immunogenic component-specific antibody is immobilised onto a solid substrate to form a first complex and the sample to be tested for immunogenic component brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-immunogenic component secondary complex, a second immunogenic component antibody, labelled with a 15 reporter molecule capable of producing a detectable signal, is then added and incubated, allowing sufficient time for the formation of a tertiary complex. Any unreacted material is washed away, and the presence of bound labelled antibody is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal or may be quantitated by comparing with a control sample.
- 20 The present invention contemplates a range of variations to the subject assay including an assay for *L. intracellularis* antibodies using, for example, recombinant peptides, polypeptides or proteins from this organism.

The solid substrate is typically glass or a polymer, the most commonly used polymers being 25 cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs or microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing the molecule to the insoluble carrier.

- 16 -

By "reporter molecule", as used in the present specification, is meant a molecule which, by its chemical nature, produces an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecule in this type of assay are either enzymes, fluorophores or 5 radionuclide containing molecules (i.e. radioisotopes). In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist which are readily available to one skilled in the art. Commonly used enzymes include horseradish peroxidase, glucose oxidase,  $\beta$ -galactosidase and alkaline phosphatase, 10 amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. It is also possible to employ fluorogenic substrates, which yield a fluorescent product.

Alternatively, fluorescent compounds, such as fluorescein and rhodamine, may be chemically 15 coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off 20 the unbound reagent, the remaining ternary complex is then exposed to the light of the appropriate wavelength, the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed. It will 25 be readily apparent to the skilled technician how to vary the procedure to suit the required purpose.

A range of genetic diagnostic assays may be employed such as polymerase chain reaction (PCR) assays, hybridisation assays or protein truncation assays. All such assays are 30 contemplated in the present invention.

- 17 -

The present invention is further described by the following non-limiting Figures and/or Examples.

In the Figures:

5

**Figure 1** is a photographic representation showing Western analysis of *L. intracellularis* antigens recognised by vaccinated pigs. Track 1 (395) was probed with pig sera from a pig (395) that had been immunised three times with the formalin killed whole *L. intracellularis* vaccine. Track 2 to 5 (Y10, Y12, Y14, Y16) were probed with sera obtained from pigs Y10, 10 Y12, Y14 and Y16, respectively on day 0.

**Figure 2** is a photographic representation of the small intestine obtained from pig Y1 on day 20.

15 **Figure 3** is a photographic representation of the small intestine obtained from pig Y2 on day 20.

**Figure 4** is a photographic representation of the small intestine obtained from pig Y4 on day 20.

20

The following single and three letter abbreviations are used for amino acid residues:

5 Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
10 Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
15 Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
20 Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
25 Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any residue	Xaa	X

- 19 -

SUMMARY OF THE SEQUENCE IDENTITY NUMBERS

SEQ ID NO.	Description
5 1	Nucleotide sequence of GroEL
2	Amino acid sequence of GroEL
3	Nucleotide sequence of GroES
4	Amino acid sequence of GroES
5	Nucleotide sequence of <i>L. intracellularis</i> component
10 6	Nucleotide sequence of <i>L. intracellularis</i> component
7	Amino acid sequence of SEQ ID NO:6
8	Nucleotide sequence of <i>L. intracellularis</i> component
9	Amino acid sequence of SEQ ID NO:8 (first coding sequence)
10	Amino acid sequence of SEQ ID NO:8 (second coding sequence)
15 11	Nucleotide sequence of <i>L. intracellularis</i> component
12	Amino acid sequence of SEQ ID NO:11
13	Nucleotide sequence of <i>L. intracellularis</i> component
14	Amino acid sequence of SEQ ID NO:13
15	Nucleotide sequence of <i>L. intracellularis</i> component
20 16	Amino acid sequence of SEQ ID NO:15
17	Nucleotide sequence of <i>L. intracellularis</i> component
18	Nucleotide sequence of <i>L. intracellularis</i> component
19	Nucleotide sequence of <i>L. intracellularis</i> component
20	Nucleotide sequence of <i>L. intracellularis</i> component
25 21	Nucleotide sequence of <i>L. intracellularis</i> component
22	Nucleotide sequence of <i>L. intracellularis</i> component
23	Nucleotide sequence of <i>L. intracellularis</i> component

- 20 -

## EXAMPLE 1

### SOURCES OF PIG TISSUE

#### Infected Pig Intestines

5 Sections of grossly thickened ilea were taken from pigs naturally or experimentally affected by PPE. The presence of *L. intracellularis* bacteria in the ilea was confirmed using immunofluorescent staining with specific monoclonal antibodies (10). An example of a suitable antibody is monoclonal antibody IG4 available from the University of Edinburgh, UK.

10

## EXAMPLE 2

### ISOLATION OF *LAWSONIA INTRACELLULARIS* BACTERIA FROM THE INFECTED PIG ILEUM

*Lawsonia intracellularis* bacteria were extracted directly from lesions of PPE in pigs by 15 filtration and further purified over a Percoll (Pharmacia, Uppsala, Sweden) gradient. Infected ilea were collected from pigs and the presence of *L. intracellularis* was confirmed histologically before storage at -80°C. Sections of ileum were thawed and approximately 8g of infected mucosa were scraped from the intestinal wall. The mucosa was homogenised with 40 ml sterile phosphate buffered saline (PBS) on half speed for 10 s using a Sorvall omnimixer. This 20 suspension was centrifuged at 2000 xg for 4 minutes. The supernatant was discarded and the cell pellet was resuspended in 40 ml PBS and re-centrifuged. This washing step was repeated twice. The cell pellet was then resuspended in 20 ml PBS and homogenised at full speed for one minute to release *L. intracellularis* bacteria.

25 This homogenate was centrifuged at 1000 xg for 4 minutes giving a pellet containing a crude mixture of homogenised epithelial cells and intestinal bacteria. The supernatant was filtered using filters with pore sized 3 µm, 1.2 µm and 0.8 µm (Millipore Corporation, MA, USA). The filtrate was centrifuged at 8000 xg for 30 minutes, resulting in a small pellet of *L. intracellularis* bacteria. The *L. intracellularis* bacteria were further purified using a 45% self forming percoll 30 gradient as follows: 2 mls of the bacterial preparation was mixed by inversion into 30 mls of

- 21 -

a 45% self forming Percoll (Pharmacia LKB, Uppsala, Sweden) gradient (45% v/v of Percoll, 150 mM NaCl). The gradients were centrifuged in a Sorval centrifuge using the SS34 rotor, at 20,000rpm for 30 minutes at 4°C. Usually a number of bands form within the gradient. The band (usually located approx. 10-20mm from the base of the tube) containing the *L. intracellularis* bacteria was collected and the volume made up to 16 mls with PBS. The solution was then centrifuged for 15 minutes at 8000rpm. The resultant pellet was washed with PBS before being resuspended in a final volume of approximately one ml.

### EXAMPLE 3

#### 10 PURIFICATION OF *LAWSONIA INTRACELLULARIS* GENOMIC DNA

Genomic DNA was extracted from percoll-gradient purified *Lawsonia intracellularis* bacteria, recovered from infected pig ilea scrapings (Example 2), by the methods described by Anderson *et al* (11) & Sambrook *et al* (12).

#### 15 EXAMPLE 4

#### IMMUNOSCREENING OF GENOMIC LIBRARIES

A lambda ZAP II *L. intracellularis* genomic library was plated on a lawn of *Escherichia coli* XLI-Blue (23) cells at a density of 2,000 plaque-forming units (pfu) per 150 mm L-broth agar plate. The library was screened with a rabbit anti- *L. intracellularis* sera using the method described in the Protoblot Technical Manual (Promega, WI, USA). Filters were blocked in a buffer containing 10mM Tris HCl, pH8.0, 150mM NaCl, 0.05% Tween 20, 1% w/w gelatin. Positive plaques identified in a primary screen were picked, replated at a lower density and rescreened until individual positive plaques were identified.

#### 25

### EXAMPLE 5

#### ISOLATION AND SEQUENCING OF cDNA INSERTS

Phagemid DNA from positive λZAP II phage clones was isolated by excision *in vivo* of the 30 pBluescript phagemid under the conditions recommended by Stratagene (CA, USA). Plasmid

- 22 -

DNA was either extracted by the method of Birnboim and Doly and the cDNA inserts sequenced by the chain termination method (21), or by the PEG-precipitation method and cycle-sequenced by the dye-terminator method, as recommended by the manufacturer (Applied Biosystems).

5

#### EXAMPLE 6

##### ANTISERA

Antisera to *L. intracellularis* bacteria were raised in rabbits and pigs. Rabbits were injected 10 intramuscularly with a preparation of Percoll gradient-purified *L. intracellularis* bacteria mixed with a double-emulsion made by processing with oil adjuvant (Freund's incomplete adjuvant, CSL Limited, Melbourne, Australia), and then with Tween 80 enhancer. Two 3 ml injections, containing 9 mg protein, were given four weeks apart. Blood samples were collected from the marginal ear vein prior to immunisation and two weeks following the second injection.

15

A 6-week old pig (395) was hyperimmunised by intramuscular injection of Percoll gradient purified *L. intracellularis* bacteria prepared with Freund's incomplete adjuvant as for the rabbit. Three injections of the prepared antigen were administered four weeks apart, and blood was collected from the jugular vein two weeks following the final injection. Diluted pig sera (1 ml, 20 1 in 200) were pre-absorbed with 100 µl *E. coli* DH5 $\alpha$  (24) lysate for 1 h at room temperature with gentle mixing. The lysate was prepared by freeze-thawing a suspension of *E. coli* in PBS.

#### EXAMPLE 7

##### SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL

25

##### ELECTROPHORESIS (SDS-PAGE)

Protein samples were resuspended in 50 µl of sample buffer (62.4 mM HCl, 2% w/v SDS, 10% v/v glycerol, 5% v/v 20 mercaptoethanol, 0.002% bromophenol blue, pH 6.8) and heated to 95°C for 5 minutes before separating solubilised proteins electrophoretically on a 0.1% w/v 30 SDS-12% w/v PAGE vertical slab gel (13).

- 23 -

**EXAMPLE 8**  
**WESTERN BLOTTING**

Proteins were electrophoretically transferred to Immobilon-P (Millipore Corporation, MA, USA) membranes in a Trans-Blot Cell (BioRad, CA, USA) at 100 V for 1 h in a buffer containing CAPS (3-[Cyclohexylamino]-l-propanesulfonic acid, pH 11, Sigma, MI, USA) and 10% v/v methanol. The membranes were then blocked with 5% w/v Blotto (Diploma skim milk powder, Melbourne, Australia) in PBS for 30 min at room temperature with gentle rocking. The filters were then transferred to antisera diluted in 5% w/v Blotto, PBS. Pre-  
10 absorbed pig antisera was diluted 1 in 200. The filters were incubated in pig antisera for 1 h followed by washing three times in PBST.

HRP conjugated anti-swine immunoglobulins (DAKO, CA, USA) were applied at a dilution of 1:2000. Enhanced Chemiluminescence (ECL, Amersham, IL, USA) was used to  
15 discriminate *L. intracellularis* proteins. Prior to ECL detection, blots were washed three times for 7 minutes each. The filters were exposed to autoradiographic film (Agfa, NJ, USA) for less than 1 minute before developing.

20 **EXAMPLE 9**  
**IDENTIFICATION OF GroEL AND GroES**

Clones found to be positive according to the immunoscreening method described in Example 4 were sequenced using the protocol detailed in Example 5. One clone isolated represented the GroEL protein. The nucleotide sequence and corresponding amino acid sequence of GroEL  
25 are shown in SEQ ID NO:1 and SEQ ID NO:2. Another clone isolated represented the GroES protein. The nucleotide sequence of GroES and corresponding amino acid sequence are shown in SEQ ID NO:3 and SEQ ID NO:4.

- 24 -

### EXAMPLE 10

#### IMMUNOFLORESCENT DETECTION OF *LAWSONIA INTRACELLULARIS* BACTERIA IN PIG FAECES

5 Faecal swabs of pigs were taken using a cotton tipped swab and then the sample was smeared onto a glass slide. After allowing ten minutes for air drying the smears were heat fixed by heating to 60°C for approximately 10 seconds. The slides were then rinsed in PBS. An amount of 30 $\mu$ l of a 1/200 dilution of a mouse ascites containing IG4 monoclonal antibody (see Example 1) was added, a glass cover slip applied, and the slides were incubated at room 10 temperature for 40 minutes. The cover slip was removed and the slides were washed (PBST for 7 minutes, three times). An amount of 30 $\mu$ l of a 1/40 dilution of a FITC conjugated anti-mouse antiserum (Silenus, Melbourne Australia) was added, a glass cover slip applied and the slides were incubated at room temperature for 40 minutes. The cover slip was removed and the slides were washed (PBST for 7 minutes X3). The slides were given a final rinse in PBS. A drop of 15 10% v/v glycerol PBS was added and a glass cover slip applied. The fluorescent bacteria were visualised under highpower (X1200) at 340 nm using a Lietz laborlux S microscope. Twenty fields were counted and the results (see Table 1) were expressed as the average number of *L. intracellularis* bacteria per high powered field.

20

### EXAMPLE 11

#### FORMALIN-KILLED *L. INTRACELLULARIS* VACCINE

The percoll gradient purified bacterial *L. intracellularis* pellet was resuspended in 1 ml of 1% formalin in saline and incubated overnight at 4°C. The percoll gradient-purified *L. intracellularis* bacteria was then mixed into a double-emulsion made by processing with oil adjuvant (Freund's incomplete adjuvant, Commonwealth Serum Laboratories, Melbourne, Australia), and then with Tween 80 enhancer.

- 25 -

**EXAMPLE 12**  
**VACCINATION PROTOCOL**

5 Twelve weaned pigs (Landrace crossed with Large White) were sourced from a Pig Improvement Company piggery and treated with Neo-Terramycin (0.25 g/kilo) for 5 days. Seven days later (day -40) pigs Y10, Y12, Y14 and Y16 were vaccinated as described. Pigs Y3, Y11 and Y13 were treated for abscess with long acting terramycin on day -34.

10 The twelve pigs were divided into three groups and treated as follows:

*Group 1 Infected Controls*

Four pigs (Ear Tag No Y1-Y4) were housed with vaccinated pigs.

15 *Group 2 Whole Bacteria Vaccine*

Four pigs (Ear Tag No. Y10, Y12, Y14 and Y16) were immunised with 0.5 ml formalin killed *L. intracellularis* bacteria emulsified in 0.5 ml of PBS/Freunds incomplete adjuvant on days -33 and -12.

20 *Group 3 Uninfected Controls*

Four pigs (Ear Tag No. Y9, Y11, Y13 and Y15) received no treatments and were housed in a separate area from the vaccinated pigs and infected control pigs.

25 **ORAL CHALLENGES OF INFECTED PIGS**

Infected ilea were collected from pigs as described in Example 1 and the presence of *L. intracellularis* was confirmed histologically before storage at -80°C. Sections of ileum were thawed and approximately 150g of infected mucosa was scraped from the intestinal wall. The 30 mucosa was homogenised with an equal volume of sterile PBS on half speed for 20 s using a

- 26 -

Sorvall omnimixer. This suspension was diluted two fold with sterile PBS to form the challenge suspension.

On day 0 each pig from Groups 1 and 2 was dosed with a 5% w/v with Na Bicarbonate solution 5 (10 ml/kg) followed by 30 ml of the challenge suspension. This was repeated on day 1 and day 2.

From day 11 onwards, the number of *L. intracellularis* bacteria in each pig's faeces was monitored by immunofluorescence. Pigs were monitored for signs of disease and shedding of 10 *L. intracellularis* bacteria. Pigs shedding greater than 100 bacteria per high powered field and scouring were killed for ethical reasons.

On day 22 the surviving pigs were humanely killed and the small intestines were recovered. Two sections of small intestine were removed 5 cms and 17 cms proximally from the ileocaecal 15 junction. These sections were fixed in 10% v/v formalin, wax embedded and sections were sent to an independent veterinary pathologist for analysis.

#### EXAMPLE 14

##### *LAWSONIA INTRACELLULARIS* PROTEINS RECOGNISED BY VACCINATED PIGS

20

Antibodies raised by pigs to *L. intracellularis* proteins post vaccination were analysed by Western blotting followed by ECL (Amersham, IL, USA) detection as described in Example 8. The results are shown in Figure 1. Vaccinated pigs produce antibodies to a range of *L. intracellularis* proteins. The most immunodominant proteins recognised are approximately 62.7 25 Kda, 58.7 Kda, 57.2 Kda, 44 Kda, 36.7 Kda and two smears from 24-26 Kda and 22-23.5 Kda. Minor immunoreactive bands had approximately the following molecular weights 67 Kda, 52.5 Kda, 50.5 Kda, 50 Kda, 48.2 KDa, 47.9 Kda, 44.7 Kda, 43.5 Kda, 42.5 Kda, 41.5 Kda, 40.5 Kda, 39 Kda, 35.3 Kda, 17 Kda, 15.5 Kda, 12 Kda and 7 Kda. The molecular weight of the proteins recognised will vary by up to 5% depending on the method used for estimation.

30

- 27 -

### EXAMPLE 15

#### SHEDDING OF *L. INTRACELLULARIS* BACTERIA BY PIGS DURING TRIAL

Three of the pigs from Group 1 (Infected control) in Example No. 12 (Y1, Y2 and Y4) shed greater than 100 *L. intracellularis* bacteria per high powered field in their faeces by day 19 post oral challenge (Table 1). Two of these pig (Y2 and Y4) had a bloody scour. All three pigs were humanely killed on day 20. Y3 shed low levels of *L. intracellularis* bacteria during the course of the infection trial. Maximal bacterial shedding for Y3 was 16 bacteria per high powered field.

10

All pigs in group 3 vaccinated with whole bacteria as set out in Example 12, never shed more than 3 *L. intracellularis* bacteria per high powered field. Vaccination with the formalin killed *L. intracellularis* vaccine reduced total bacterial shedding of *L. intracellularis* bacteria by vaccinated pigs by 98.5% when compared with group 1 pigs.

15

None of the group 3 pigs (uninfected controls) shed any *L. intracellularis* bacteria during the course of the trial.

The results of shedding of *L. intracellularis* bacteria per pig are shown in Table 1.

20

### EXAMPLE 16

#### GROSS PATHOLOGY FOR TRIAL A

##### *Group 1      Infected Controls*

25 Y1      Approximately 5 cm of terminal ileum was grossly thickened. No other signs of PPE were evident macroscopically. Findings are consist with intestinal adenomatosis (See Figure 2).

Y2      The intestine was found to be grossly thickened and the serosa had the characteristic cerebriform forms (Figure 3). Over 2.5 metres of the intestine was involved. The lumen 30      of the intestine was found to contain fresh blood and fibrinous casts were evident.

- 28 -

**Proliferative haemorrhagic enteropathy.**

Y3 No gross signs of PPE were evident.  
Y4 The intestine was found to have necrotic enteritis (Figure 4). The mucosal surface was replaced with a fibrinous pseudomembrane. Oedema of the mesentery was clearly 5 evident. Over 2.0 meters of intestine was involved.

**Group 2 Whole *L. intracellularis* cell vaccine**

Y10 No gross signs of PPE.  
Y12 No gross signs of PPE.  
10 Y14 No gross signs of PPE.  
Y16 No gross signs of PPE.

**Group 3 Uninfected controls**

Y9 No gross signs of PPE.  
15 Y11 No gross signs of PPE.  
Y13 No gross signs of PPE.  
Y15 No gross signs of PPE.

**EXAMPLE 17**

**20 HISTOPATHOLOGY REPORT FOR TRIAL**

Reports are based on established histopathological descriptions in Jubb *et al* (20).

**Group 1 Infected control group**

25 Y1 Numerous microfocal/confluent lesions of Porcine Intestinal Adenomatosis (PIA) are associated with Peyer's Patches.  
Y2 Serious generalised (annular) lesions of Porcine Intestinal Adenomatosis.  
Y3 No conclusive evidence of PIA. Sparse microfocal lesions suggestive of a non-specific mild reactive ( reparational ) hyperplasia ( rather than an adenomatosis ).  
30 Y4 Severe generalised (annular) lesions of PIA.

- 29 -

*Group 2 Whole L. intracellularis cell vaccine*

- Y10 No conclusive evidence of PIA.
- Y12 No conclusive evidence of PIA.
- 5 Y14 No conclusive evidence of PIA.
- Y16 No conclusive evidence of PIA. Possible single microfocus of PIA is associated with Peyers Patch.

*Group 3 Uninfected controls*

- 10 Y11 No conclusive evidence of PIA.
- Y9 No conclusive evidence of PIA.
- Y13 Intestine was not recovered since pig was killed due to lameness at day 15.
- Y15 Diagnosis not possible because of the poor quality sections.

15

**EXAMPLE 18**

IMMUNOSCREENING OF A *L. INTRACELLULARIS* LIBRARY USING  
EXPERIMENTAL SERA FROM VACCINATED PIGS

- 20 *L. intracellularis* genomic DNA was purified as described in Example 3. The DNA was partially digested with the restriction endonuclease *Sau*3A (Promega) and ligated into Lambda ZAP II Express (Stratagene). The lambda library was plated on a lawn of *E. coli* XLI-Blue cells at a density of 10,000 pfu per 150 Mm L-broth agar plate. The library was screened, as described in Example 4, with sera from Y12. The pig Y12 was immunised with formalin killed
- 25 *L. intracellularis*, as described in Example 11 & 12. Vaccinated pigs produced antibodies to a range of *L. intracellularis* proteins, as described in Example 14. A number of phage clones expressing *L. intracellularis* proteins were identified.

- 30 -

### EXAMPLE 19

#### ANALYSIS OF *L. INTRACELLULARIS* EXPRESSING PHAGE CLONES

5 Phagemid DNA from positive λZAP II Express phage clones was isolated by *in vivo* excision, by the conditions recommended by the manufacturer (Stratagene). Plasmid DNA, for restriction analysis was extracted by alkaline-lysis, as described by Sambrook *et al* (12), and for automated sequencing, using the High Pure Plasmid Kit, as recommended by the manufacturer (Boehringer Mannheim). DNA sequencing of inserts was performed by the Dye-  
10 terminator method of automated sequencing (ABI Biosystems). The sequences identified are set out in SEQ ID NOS: 5-23 (see Example 20).

### EXAMPLE 20

#### IDENTIFICATION OF *L. INTRACELLULARIS* COMPONENTS

15 Sequence similarity of the DNA molecules encoding putative vaccine candidates identified from Example 18 and 19, was identified using BLAST (27). Nucleotide sequence SEQ ID NO:6 and its corresponding amino acid sequence SEQ ID NO:7 have sequence similarity to flagellar basal body rod protein. SEQ ID NO:8 (nucleotide) and SEQ ID NOS:9 and 10  
20 (amino acid) have sequence similarity to autolysin. SEQ ID NO:11 (nucleotide) and SEQ ID NO:12 (amino acid) show sequence similarity to S-adenosylmethionine: tRNA ribosyltransferase-isomerase (queuosine biosynthesis protein queA).

SEQ ID NO:13 (nucleotide) and SEQ ID NO:14 (amino acid) show sequence similarity to  
25 enoyl-(acyl-carrier-protein) reductase. SEQ ID NO:15 (nucleotide) and SEQ ID NO:16 (amino acid) show sequence similarity to a glucarate transporter. Other nucleotide sequences encoding putative vaccine candidates are SEQ ID NO:5, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 and SEQ ID NO:23.

30 Those skilled in the art will appreciate that the invention described herein is susceptible to

- 31 -

variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more  
5 of said steps or features.

- 32 -

**TABLE 1**

**32A**

**32B**

- 32A -

TABLE I

Vaccination	Challenge
-------------	-----------

	Day -40	Day -33	Day -26	Day -12	Day 0	Day 1	Day 2	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22
1 infected controls								1+	1+	0	0	5+	10+	50+	100+	15			
2 infected controls								0	1+	1+	1+	3+	1+	70+	100+	100	PHE 2.5 M		
3 infected controls								0	0	0	0	0	1+	4	16	1+	0	1	
4 infected controls								1+	0	0	10+	0	5+	60+	200+	80	PHE 2.0 M		
10 whole bugs								0	0	0	0	0	1+	1+	0	0	0	0	0

- 32B -

12 whole bugs	1 ml killed whole cell 1 ml killed whole cell	1+	0	0	0	2+	0	0	0	0	0	0
14 whole bugs	1 ml killed whole cell 1 ml killed whole cell	0	0	0	0	1+	0	<1	<1	0	0	0
16 whole bugs	1 ml killed whole cell 1 ml killed whole cell	0	0	0	0	0	0	3	<1	0	0	0
9 Uninfected controls		0	0	0	0	0	0	0	0	0	0	0
11 Uninfected controls		0	0	0	0	0	0	0	0	0	0	0
13 Uninfected controls		0	0	0	0	0	0	0	0	0	0	0
15 Uninfected controls		0	0	0	0	0	0	0	0	0	0	0

**BIBLIOGRAPHY**

- 1 Barker, I.K. and Van Dreumel, A.A. (1985) In "Pathology of Domestic Animals," 3rd Edition, Vol. 2 p. 1-237, eds K.V.F. Jubb, P.C. Kennedy and N. Palmer. (Academic Press: Orlando).
- 2 Rowland, A.C. and Lawson, G.H.K. (1976) *Veterinary Record* 97:178-180.
- 3 Love, R.J. and Love, D.M. (1977) *Veterinary Record* 100:473
4. Jonsson, L. and Martinsson, K. (1976) *Acta Veterinaria Scandinavica* 17:223-232.
5. O'Neil, I. P.A. (1970) *Veterinary Record* 87:742-747.
6. Straw, B.E. (1990). *Journal of American Veterinary Medical Association* 197: 355-357.
7. Stills, H.F. (1991). *Infection and immunology* 59: 3227-3236.
8. Gebhert, C.J., Ward, G.E., Chang, K. And Kurtz, H.J. (1983). *American Journal of Veterinary Research* 44:361-367.
9. Lawson, G.H.K., McOrist, S., Jansi, S. and Mackie, R.A. (1993) *Journal of Clinical Microbiology* 31:1136-1142.
10. McOrist, S., Boid, R., Lawson, G.H.K. and McConnell, I. (1987) *The Veterinary Record* 121:421-422.
11. Anderson, B.J., M.M. Bills, J.R. Egerton, and J.S. Mattick. (1984) *Journal of Bacteriology* 160:748-754.

- 34 -

12. Sambrook, J., E.F. Fritsch, and T. Maniatis. (1989) Molecular cloning. A laboratory manual. Second edition. Cold Spring Harbour Laboratory, Cold Spring Harbour, N.Y.
13. Laemmli, U.K. (1970) *Nature* 227: 680-685.
14. McOrist, S, Jasni, S, Mackie, RA, MacIntyre, N, Neef, N. and Lawson GHK (1993) *Infection and Immunity* 61: 4286-4292.
15. Fox, JG, Murphy, JC, Otto, G Pecquet-Goad, ME, Larson, QHK and Scott JA (1989) *Veterinary Pathology* 26: 515-517.
16. Elwell, MR, Chapman, AL and Frenkel, JK (1981) *Veterinary Pathology* 18: 136-139.
17. Schodek, TR and Fox JG (1990) *Veterinary Pathology* 27: 73-80.
18. Mason, RW, Monkton, P and Hasse D (1995) *Australian Veterinary Journal* (in press).
19. Manthorpe, M, Cornefert-Jensen, F., Hartikka, J., Felgner, J, Rundell, A, Margalith, M and Dwarki, V. (1993) *Human Gene Therapy* 4: 419-431.
20. Jubb KVC, Kennedy, PC and Palmer, NC (1993). The Pathology of Domestic Animals 4th ed. San Diego, CA, Academic Press pp 229-233.
21. Birnboim, HC and Doly J (1979) *Nucleic Acids Research* 7: 1513.
22. Sanger, F, Nicklen, S and Coulson, AR (1977) *Proceedings of the National Academy of Science* 74: 5463.

- 35 -

23. Block, WO, Fernandes, JM and Short, JM (1987) *Biotechnology* 5: 376-79.
24. Woodcock, DM *et al* (1989) *Nucleic Acids Research* 17: 3469-78.
25. Studier, FW *et al* (1990) *Methods in Enzymology* 185: 60-89.
26. McOrist, S *et al* (1995) *International Journal of Systematic Bacteriology* 45: 820-825.
27. Gish, W and States, D.J. (1993) *Nature Genetics* 3: 266-272.

- 36 -

## SEQUENCE LISTING

### (1) GENERAL INFORMATION:

(i) APPLICANT: (OTHER THAN US) DARATECH PTY LTD and PIG RESEARCH  
(US ONLY): MICHAEL PANACCIO and DETLEF HASSE

(ii) TITLE OF INVENTION: THERAPEUTIC AND DIAGNOSTIC  
COMPOSITIONS

(iii) NUMBER OF SEQUENCES: 23

### (iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: DAVIES COLLISON CAVE  
(B) STREET: 1 LITTLE COLLINS STREET  
(C) CITY: MELBOURNE  
(D) STATE: VICTORIA  
(E) COUNTRY: AUSTRALIA  
(F) ZIP: 3000

### (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

### (vi) CURRENT APPLICATION DATA:

(A) PCT INTERNATIONAL  
(B) FILING DATE: 29-NOV-1996

### (vii) PRIOR APPLICATION DATA:

- 37 -

(A) APPLICATION NUMBER: PN6911/95

(B) FILING DATE: 30-NOV-1995

(A) APPLICATION NUMBER: PN6910/95

(B) FILING DATE: 30-NOV-1995

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: HUGHES DR, E JOHN L

(C) REFERENCE/DOCKET NUMBER: EJH/AF

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: +61 3 9254 2777

(B) TELEFAX: +61 3 9254 2770

- 38 -

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1647 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1647

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG GCT TCT AAA GAA ATC CTT TTT GAT GCT AAA GCC CGT GAA AAA CTT	48
Met Ala Ser Lys Glu Ile Leu Phe Asp Ala Lys Ala Arg Glu Lys Leu	
1                   5                   10                   15	
TCA CGA GGT GTA GAT AAA CTT GCA AAT GCT GTT AAA GTA ACA CTT GGA	96
Ser Arg Gly Val Asp Lys Leu Ala Asn Ala Val Lys Val Thr Leu Gly	
20                   25                   30	
CCT AAA GGC CGT AAT GTC GTT ATT GAA AAG TCT TTT GGT TCC CCA GTT	144
Pro Lys Gly Arg Asn Val Val Ile Glu Lys Ser Phe Gly Ser Pro Val	
35                   40                   45	
ATT ACA AAA GAT GGT GTA TCT GTT GCA AAA GAA ATT GAA CTT GAA GAT	192
Ile Thr Lys Asp Gly Val Ser Val Ala Lys Glu Ile Glu Leu Glu Asp	
50                   55                   60	
AAG TTT GAA AAT ATG GGC GCT CAA ATG GTT AAA GAA GTA GCT CCC AAA	240
Lys Phe Glu Asn Met Gly Ala Gln Met Val Lys Glu Val Ala Pro Lys	
65                   70                   75                   80	

- 39 -

ACT ACC GAT ATT GCT GGT GAT GGA ACT ACA ACA GCA GCA GTC CTT GCA	288		
Thr Ser Asp Ile Ala Gly Asp Gly Thr Thr Thr Ala Thr Val Leu Ala			
85	90	95	
CAA GCT ATT TAT CGT GAA GGT GTA AAA CTT GTA GCA GCT GGT CGT AAT	336		
Gln Ala Ile Tyr Arg Glu Gly Val Lys Leu Val Ala Ala Gly Arg Asn			
100	105	110	
CCT ATG GCC ATT AAA CGT GGC ATA GAT AAA GCT GTT GTT GCT GTT ACT	384		
Pro Met Ala Ile Lys Arg Gly Ile Asp Lys Ala Val Val Ala Val Thr			
115	120	125	
AAA GAA CTA AGC GAC ATT ACA AAG CCT ACT CGT GAC CAA AAA GAA ATA	432		
Lys Glu Leu Ser Asp Ile Thr Lys Pro Thr Arg Asp Gln Lys Glu Ile			
130	135	140	
GCT CAA GTT GGA ACC ATT TCT GCA AAC TCT GAT ACA ACA ATA GGT AAT	480		
Ala Gln Val Gly Thr Ile Ser Ala Asn Ser Asp Thr Thr Ile Gly Asn			
145	150	155	160
ATC ATA GCT GAA GCT ATG GCT AAA GTT GGA AAA GGA GGT GTT ATC ACA	528		
Ile Ile Ala Glu Ala Met Ala Lys Val Gly Lys Gly Val Ile Thr			
165	170	175	
GTT GAG GAA GCT AAA GGT CTT GAA ACT ACA TTA GAT GTG GTT GAA GGA	576		
Val Glu Glu Ala Lys Gly Leu Glu Thr Thr Leu Asp Val Val Glu Gly			
180	185	190	
ATG AAG TTT GAC CGT GGC TAC CTC TCT CCA TAC TTT GTA ACT AAT CCT	624		
Met Lys Phe Asp Arg Gly Tyr Leu Ser Pro Tyr Phe Val Thr Asn Pro			
195	200	205	
GAG AAA ATG GTT TGT GAA CTT GAT AAC CCT TAT ATC CTT TGT AAT GAG	672		
Glu Lys Met Val Cys Glu Leu Asp Asn Pro Tyr Ile Leu Cys Asn Glu			
210	215	220	
AAA AAG ATT ACT AGC ATG AAA GAC ATG CTA CCA ATC TTA GAA CAA GTT	720		

- 40 -

Lys Lys Ile Thr Ser Met Lys Asp Met Leu Pro Ile Leu Glu Gln Val				
225	230	235	240	
GCT AAA GTA AAC CGT CCA CTC CTT ATT ATT GCT GAA GAC GTA GAA GGT				768
Ala Lys Val Asn Arg Pro Leu Leu Ile Ile Ala Glu Asp Val Glu Gly				
245	250	255		
GAA GCA CTT GCA ACA CTT GTA GTC AAT AAG CTC CGT GGA GCA CTC CAA				816
Glu Ala Leu Ala Thr Leu Val Val Asn Lys Leu Arg Gly Ala Leu Gln				
260	265	270		
GTT GTA GCC GTA AAA GCT CCT GGT TTT GGT GAA CGC CGT AAA GCT ATG				864
Val Val Ala Val Lys Ala Pro Gly Phe Gly Glu Arg Arg Lys Ala Met				
275	280	285		
CTT GAA GAT ATT GCT ATC CTT ACT GGA GGA GAA GCA ATA TTT GAA GAT				912
Leu Glu Asp Ile Ala Ile Leu Thr Gly Gly Glu Ala Ile Phe Glu Asp				
290	295	300		
CGT GGT ATA AAG CTT GAA AAT GTA AGC TTG TCT TCT TTA GGA ACA GCT				960
Arg Gly Ile Lys Leu Glu Asn Val Ser Leu Ser Ser Leu Gly Thr Ala				
305	310	315	320	
AAA CGT GTA GTT ATT GAC AAA GAA AAT ACT ACT ATC GTT GAT GGT GCT				1008
Lys Arg Val Val Ile Asp Lys Glu Asn Thr Thr Ile Val Asp Gly Ala				
325	330	335		
GGA AAA TCA GAA GAT ATT AAA GCT CGA GTT AAA CAA ATT CGT GCA CAA				1056
Gly Lys Ser Glu Asp Ile Lys Ala Arg Val Lys Gln Ile Arg Ala Gln				
340	345	350		
ATT GAA GAA ACA AGC TCA GAT TAT GAT CGT GAA AAA CTT CAA GAA CGT				1104
Ile Glu Glu Thr Ser Ser Asp Tyr Asp Arg Glu Lys Leu Gln Glu Arg				
355	360	365		
CTT GCA AAA CTT GTT GGT GGA GTA GCT GTT ATC CAT GTT GGA GCT GCT				1152
Leu Ala Lys Leu Val Gly Gly Val Ala Val Ile His Val Gly Ala Ala				

- 41 -

370	375	380	
ACT GAA ACT GAA ATG AAA GAG AAG GAT CGT GTA GAA GAT GCT CTA Thr Glu Thr Glu Met Lys Glu Lys Lys Asp Arg Val Glu Asp Ala Leu			1200
385	390	395	400
AAT GCA ACA AGA GCT GCG GTT GAA GAA GGT ATT GTC CCT GGT GGT GGT Asn Ala Thr Arg Ala Ala Val Glu Glu Gly Ile Val Pro Gly Gly			1248
405	410	415	
ACT GCT TTT GTC CGC TCC ATT AAA GTC CTT GAT GAT ATT AAA CCT GCT Thr Ala Phe Val Arg Ser Ile Lys Val Leu Asp Asp Ile Lys Pro Ala			1296
420	425	430	
GAT GAT GAT GAA CTT GCT GGA CTT AAT ATC ATC CGT CGT TCT CTT GAA Asp Asp Asp Glu Leu Ala Gly Leu Asn Ile Ile Arg Arg Ser Leu Glu			1344
435	440	445	
GAG CCT TTA CGT CAA ATT GCT GCA AAT GCT GGC TAT GAA GGT TCT ATT Glu Pro Leu Arg Gln Ile Ala Ala Asn Ala Gly Tyr Glu Gly Ser Ile			1392
450	455	460	
GTT GTA GAA AAA GTT CGT GAA CCA AAA GAT GGT TTT GGA TTT AAT GCT Val Val Glu Lys Val Arg Glu Pro Lys Asp Gly Phe Gly Phe Asn Ala			1440
465	470	475	480
GCA TCA GGA GAA TAT GAA GAC CTT ATT AAA GCT GGT GTC ATT GAT CCT Ala Ser Gly Glu Tyr Glu Asp Leu Ile Lys Ala Gly Val Ile Asp Pro			1488
485	490	495	
AAA AAA GTT ACA CGT ATT GCA TTA CAA AAT GCA GCA TCA GTA GCC TCC Lys Lys Val Thr Arg Ile Ala Leu Gln Asn Ala Ala Ser Val Ala Ser			1536
500	505	510	
TTA CTT CTA ACT ACA GAA TGC GCT ATT GCT GAA AAA CCA GAA CCT AAA Leu Leu Leu Thr Thr Glu Cys Ala Ile Ala Glu Lys Pro Glu Pro Lys			1584
515	520	525	



- 43 -

Gln Ala Ile Tyr Arg Glu Gly Val Lys Leu Val Ala Ala Gly Arg Asn

100 105 110

Pro Met Ala Ile Lys Arg Gly Ile Asp Lys Ala Val Val Ala Val Thr

115 120 125

Lys Glu Leu Ser Asp Ile Thr Lys Pro Thr Arg Asp Gln Lys Glu Ile

130 135 140

Ala Gln Val Gly Thr Ile Ser Ala Asn Ser Asp Thr Thr Ile Gly Asn

145 150 155 160

Ile Ile Ala Glu Ala Met Ala Lys Val Gly Lys Gly Gly Val Ile Thr

165 170 175

Val Glu Glu Ala Lys Gly Leu Glu Thr Thr Leu Asp Val Val Glu Gly

180 185 190

Met Lys Phe Asp Arg Gly Tyr Leu Ser Pro Tyr Phe Val Thr Asn Pro

195 200 205

Glu Lys Met Val Cys Glu Leu Asp Asn Pro Tyr Ile Leu Cys Asn Glu

210 215 220

Lys Lys Ile Thr Ser Met Lys Asp Met Leu Pro Ile Leu Glu Gln Val

225 230 235 240

Ala Lys Val Asn Arg Pro Leu Leu Ile Ile Ala Glu Asp Val Glu Gly

245 250 255

Glu Ala Leu Ala Thr Leu Val Val Asn Lys Leu Arg Gly Ala Leu Gln

260 265 270

Val Val Ala Val Lys Ala Pro Gly Phe Gly Glu Arg Arg Lys Ala Met

275 280 285

Leu Glu Asp Ile Ala Ile Leu Thr Gly Gly Glu Ala Ile Phe Glu Asp

- 44 -

290

295

300

Arg Gly Ile Lys Leu Glu Asn Val Ser Leu Ser Ser Leu Gly Thr Ala  
305 310 315 320

Lys Arg Val Val Ile Asp Lys Glu Asn Thr Thr Ile Val Asp Gly Ala  
325 330 335

Gly Lys Ser Glu Asp Ile Lys Ala Arg Val Lys Gln Ile Arg Ala Gln  
340 345 350

Ile Glu Glu Thr Ser Ser Asp Tyr Asp Arg Glu Lys Leu Gln Glu Arg  
355 360 365

Leu Ala Lys Leu Val Gly Gly Val Ala Val Ile His Val Gly Ala Ala  
370 375 380

Thr Glu Thr Glu Met Lys Glu Lys Lys Asp Arg Val Glu Asp Ala Leu  
385 390 395 400

Asn Ala Thr Arg Ala Ala Val Glu Glu Gly Ile Val Pro Gly Gly  
405 410 415

Thr Ala Phe Val Arg Ser Ile Lys Val Leu Asp Asp Ile Lys Pro Ala  
420 425 430

Asp Asp Asp Glu Leu Ala Gly Leu Asn Ile Ile Arg Arg Ser Leu Glu  
435 440 445

Glu Pro Leu Arg Gln Ile Ala Ala Asn Ala Gly Tyr Glu Gly Ser Ile  
450 455 460

Val Val Glu Lys Val Arg Glu Pro Lys Asp Gly Phe Gly Phe Asn Ala  
465 470 475 480

Ala Ser Gly Glu Tyr Glu Asp Leu Ile Lys Ala Gly Val Ile Asp Pro  
485 490 495

- 45 -

Lys Lys Val Thr Arg Ile Ala Leu Gln Asn Ala Ala Ser Val Ala Ser

500

505

510

Leu Leu Leu Thr Thr Glu Cys Ala Ile Ala Glu Lys Pro Glu Pro Lys

515

520

525

Lys Asp Met Pro Met Pro Gly Gly Met Gly Gly Met Gly Gly Met

530

535

540

Asp Gly Met Tyr

545

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..306

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG AAC CTG AAA CCT TTG AAT GAC CGT GTT TTA GTA AAA CGT CTT GAA

48

Met Asn Leu Lys Pro Leu Asn Asp Arg Val Leu Val Lys Arg Leu Glu

1

5

10

15

TCT GAA GAA AAA ACA GCT GGT GGA CTC TAT ATC CCT GAT ACT GCT AAA

96

Ser Glu Glu Lys Thr Ala Gly Gly Leu Tyr Ile Pro Asp Thr Ala Lys

20

25

30

- 46 -

GAA AAA CCA TCT CGT GGT GAA GTT GTT GCT GGT GGA CCT GGT AAA CAT	144	
Glu Lys Pro Ser Arg Gly Glu Val Val Ala Val Gly Pro Gly Lys His		
35	40	45
ACA GAT GAT GAT GGT AAA TTA ATA CCT ATG GCT GTA AAA GCA GGA GAT ACA	192	
Thr Asp Asp Gly Lys Leu Ile Pro Met Ala Val Lys Ala Gly Asp Thr		
50	55	60
GTT CTT TTT AAT AAG TAT GCA GGA ACA GAA GTA AAG CTT GAT GGT GTA	240	
Val Leu Phe Asn Lys Tyr Ala Gly Thr Glu Val Lys Leu Asp Gly Val		
65	70	75
80		
GAG CAT CTA GTT ATG CGT GAA GAT GAC ATC CTA GCT GTT ATT ACT GGA	288	
Glu His Leu Val Met Arg Glu Asp Asp Ile Leu Ala Val Ile Thr Gly		
85	90	95
GAA ACT GGC CGC AAG TGA	306	
Glu Thr Gly Arg Lys *		
100		

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 101 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asn Leu Lys Pro Leu Asn Asp Arg Val Leu Val Lys Arg Leu Glu			
1	5	10	15

Ser Glu Glu Lys Thr Ala Gly Gly Leu Tyr Ile Pro Asp Thr Ala Lys

- 47 -

20

25

30

Glu Lys Pro Ser Arg Gly Glu Val Val Ala Val Gly Pro Gly Lys His

35

40

45

Thr Asp Asp Gly Lys Leu Ile Pro Met Ala Val Lys Ala Gly Asp Thr

50

55

60

Val Leu Phe Asn Lys Tyr Ala Gly Thr Glu Val Lys Leu Asp Gly Val

65

70

75

80

Glu His Leu Val Met Arg Glu Asp Asp Ile Leu Ala Val Ile Thr Gly

85

90

95

Glu Thr Gly Arg Lys

100

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4972 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AACTCCTGGT CTATCAAGAT CAACTAAAAA ATATTCTTTA TCTAATAGTT	50
GCTAAAAAT AATTGTACCT ACAGGTAAAT GAAGAATCAA ATCTTCCCCT	100
TTTTTACCAT GACGCTGGCT CCCTTTACCA CCTTCTCCAT TTTGAGCTCT	150
ATAGTGACGT TGCACACGAA AATCATAAAAG GGTAAACAAA CGTGAATCAG	200
CTTTAAAAAT TATATTACCT CCATCTCCTC CATCCCCCTCC ATTAGGTCCA	250

- 48 -

CCTTTAGGTA TAAACTTTC GCGTCTAAAT GAAACACATC CATTCCACC	300
TTTCCTGCG CTCACGCTAA TAGTTACTTC ATCAACAAAA CGCATGATTA	350
TCCTTCAAT AACAAATATC TATTCAATAC TGTTACTAAC TTGTTTACTG	400
TTTTTCTAG AAAATTACCT GGCTAATTAT TATAGTTATA TCTAGATTAA	450
TGAAAAGGA AGAAGTCATT ACACCTCTTC CTTATTAATA GAATCCTGGA	500
ATAATTATTA TACGGTGGGT TGTATATGCA CTCTACTATA TCTTTTACAT	550
TTACGAAAAT ATGTTTCATA AGTTACTATA CCATTAACCT TTGCAAATAA	600
AGTATAGTCT CTTCCCATTTC CAACATTTTC TCCAGGATGA ATTTTTGTAC	650
CTAGTTGACG AACAGGATA TTGCCTGCCA AGACTTTCTG GCCGCCGAAA	700
CGCTTATAC CACGACGTTG TCCTGGACTA TCTCTACCAT TGCGAGAACT	750
TCCACCAGCT TTCTTATGGG CCATTTTAAT ATCTCTTAA AGCTGAATAC	800
CTGTTACTTT TAGAGCTGTA TAGTCTTGAC GATGACCTTG GAGTTTACGT	850
GAGTCATTT TCCTCCACTT TTTAAAAACA AGAATTTTTT TATCACGACC	900
ATGCTCAAGA ACTTTAGCTA TAACTTTAGC ATTATTAATA TATGGTGTTC	950
CAATTTGAGG AGATGAACCA CCAATCATAA AAATTTTATC AAAAAAAATT	1000
TCTGTTCCAA CTTCAGCGTC TATTTAGAA ACAAAAATTT TAGAACCTC	1050
TTCAACACAG AATTGTTTC CACCAAGCTTC AATAATTGCG TACATAAATA	1100
ATGTGCCTCC CAAAAAAGAC AAGAAATACT AATTTGATAT TTCAATATT	1150
GTCAAGTAGG AACTTTATCT TTAGAATGTT AGATGTAACA ATTTTTTAG	1200
AAAAAAAATA TTTTCAATAC AATAGGAAAA GAGGAAAAAA AAAAAAGATTT	1250
TTAGAAAAAA TTTTTATTTC TCCAAAAAAT GCAAAAATAT AAAAAATTCT	1300
AATAGGATAG AAGTTATTAC TGTATTGATT TTCAAGACTT ACTTAAAAT	1350
TTTTATAAAA AAATTTGCAT TCCCCCTCTTC CCAATTCCCA TAGAGAAGAT	1400
TATTTATCCT AACGATTGGT GGACGCTAAG TCCCTGCTGT TTTGATTATA	1450
TATCAAATGT TGAAACAAAT TTTGTTTAGT TTCTTTTGT ACTCTAAAAA	1500
GAAGACAAAA AATTCTTTAT AAACTGTACA CTCTAAACAA AATAGTTCAC	1550
AATAAACAGC AATACATTAT AATTAAATTGG AGGATACTAT TGTATGAAC	1600
CTGAAACCTT TGAATGACCG TGTGTTAGTA AAACGCTTGT AATCTGAAGA	1650
AAAAACAGCT GGTGGACTCT ATATCCCTGA TACTGCTAAA GAAAACCAT	1700
CTCGTGGTGA AGTTGTTGCT GTTGGACCTG GTAAACATAC AGATGATGGT	1750
AAATTAATAC CTATGGCTGT AAAAGCAGGA GATACAGTTC TTTTTAATAA	1800
GTATGCAGGA ACAGAAGTAA AGCTTGATGG TGTAGAGCAT CTAGTTATGC	1850
GTGAAGATGA CATCCTAGCT GTTATTACTG GAGAAACTGG CCGCAAGTGA	1900
AAAAGGCGTA AATAAAAAGA TCGGTGATCT TTAATAATTT TATTCAAGTTA	1950
TAATGAAAAC ACTAATTACA CGCACTCTCT GAGAATTTTC TCAGAAAAC	2000
ATATTTAACCA ATTCTAAAAT CGATATGTTT TTAGGAGGAA AACCCCTAATG	2050
GCTTCTAAAG AAATCCTTT TGATGCTAAA GCCCGTAAA AACTTTCAAG	2100

- 49 -

AGGTGTAGAT AAACTTGCAA ATGCTGTTAA AGTAACACTT GGACCTAAAG	2150
GCCGTAATGT CGTTATTGAA AAGTCCTTG GTTCCCCAGT TATTACAAAA	2200
GATGGTGTAT CTGTTGCAA AGAAAATTGAA CTTGAAGATA AGTTTGAAAA	2250
TATGGGCGCT CAAATGGTTA AAGAAGTAGC TCCCAAAATC AGCGATATTG	2300
CTGGTGATGG AACTACAACA GCAACAGTCC TTGCACAAGC TATTTATCGT	2350
GAAGGTGTAA AACTTGTAGC AGCTGGTGT AATCCTATGG CCATTAAACG	2400
TGGCATAGAT AAAGCTGTTG TTGCTGTTAC TAAAGAACTA AGCGACATTA	2450
CAAAGCCTAC TCGTGACCAA AAAGAAATAG CTCAGTTGG AACCATTCT	2500
GCAAACCTCTG ATACAACAAT AGGTAATATC ATAGCTGAAG CTATGGCTAA	2550
AGTTGGAAAA GGAGGTGTAA TCACAGTTGA GGAAGCTAAA GGTCTTGAAA	2600
CTACATTAGA TGTGGTTGAA GGAATGAAGT TTGACCGTGG CTACCTCTCT	2650
CCATACCTTG TAACTAATCC TGAGAAAATG GTTGTGAAC TTGATAACCC	2700
TTATATCCTT TGTAATGAGA AAAAGATTAC TAGCATGAAA GACATGCTAC	2750
CAATCTTAGA ACAAGTTGCT AAAGTAAACC GTCCACTCCT TATTATTGCT	2800
GAAGACGTAG AAGGTGAAGC ACTTGCAACA CTTGTAGTCA ATAAGCTCCG	2850
TGGAGCACTC CAAGTTGTAG CCGTAAAAGC TCCTGGTTT GGTGAACGCC	2900
GTAAAGCTAT GCTTGAAGAT ATTGCTATCC TTACTGGAGG AGAACATA	2950
TTTGAAGATC GTGGTATAAA GCTTGGAAAT GTAAGCTTGT CTTCTTTAGG	3000
AACAGCTAAA CGTGTAGTTA TTGACAAAGA AAATACTACT ATCGTTGATG	3050
GTGCTGGAAA ATCAGAAGAT ATTAAGCTC GAGTTAAACA AATTCTGCA	3100
CAAATTGAAG AAACAAGCTC AGATTATGAT CGTAAAAAC TTCAAGAACG	3150
TCTTGCAAAA CTTGTTGGTG GAGTAGCTGT TATCCATGTT GGAGCTGCTA	3200
CTGAAACTGA AATGAAAGAG AAGAAGGATC GTGTAGAAGA TGCTCTAAAT	3250
GCAACAAGAG CTGCGGTTGA AGAAGGTATT GTCCCTGGTG GTGGTACTGC	3300
TTTGTCCGC TCCATTAAG TCCTTGATGA TATTAAACCT GCTGATGATG	3350
ATGAACCTGC TGGACTTAAT ATCATCCGTC GTTCTCTTGA AGAGCCTTTA	3400
CGTCAAATTG CTGCAAATGC TGGCTATGAA GGTCTATTG TTGTAGAAAA	3450
AGTTCTGAA CCAAAAGATG GTTTGGATT TAATGCTGCA TCAGGAGAAT	3500
ATGAAGACCT TATTAAAGCT GGTGTCATTG ATCCTAAAAA AGTTACACGT	3550
ATTGCATTAC AAAATGCAGC ATCAGTAGCC TCCTTACTTC TAACTACAGA	3600
ATGCGCTATT GCTGAAAAAC CAGAACCTAA AAAAGATATG CCTATGCCCTG	3650
GCGGTGGTAT GGGTGGTATG GGTGGTATGG ACGGTATGTA CTAGTCCTAT	3700
CTTCAGTACA ACCTAGATGT ATAAAAACCC CAGAACGAAAT GCTTCCGGGG	3750
TTTTTATCTT TCAGCATAAA AAATTAATAT TTAATATACA GACACATTAT	3800
TTTGGTATTT ATTATTTATT ATGATCAAAT ATATAGACTG GATACAAAAA	3850
ACAACAATGA TGTTTAAAAA GGCAGGGATA GATTCAACCAA AACTCTCTGC	3900
AGAAACTTATA TTAAGTCATG TTTAAATAT TACACGATTA CAAATAATAA	3950

- 50 -

TGACTCCTTT	TGAACCTATT	CCAACTAATA	GCTACTCAAC	GCTTAATGAT	4000
ATCATGTTAA	GAAGACTCCA	TGGAGAACCA	ATTGCATATTC	TCACAGGGAA	4050
AAAAGAATT	TTTCACGAG	AATTAAAGT	CACTCAAGCC	ACACTTATCC	4100
CTCGCCCAGA	GACAGAGTTA	CTTATAGAAT	TTGTATTAAA	CCATATTAAC	4150
CCAAACACAAC	AAATATACTT	TGCAGACTTA	GGTACAGGTA	GTGGGTGTAT	4200
TGCAATTACA	CTAGCTGCTG	AAAGAAAAAA	TTGGTTAGGT	ATTGCTACTG	4250
ATATCTCTAG	TGAAGCATT	AAAATAGCTA	AACTTAATAG	TTTAAAAAAAT	4300
AACACTCATA	GTCAACTACA	GTTTCTCAA	TCAGATTTA	CACAACCACT	4350
CTGTCTACCC	TCTTCATTAG	ACTTATATAT	CAGTAATCCT	CCATATATAA	4400
GTGAAAATGA	ACTGACCTCT	CTTCCGCATG	AAGTAATATC	TTTGAAACCT	4450
AAAATAGCTC	TTACACCACA	AAAATGTATT	CATCTTGATG	AAATAAAATAC	4500
CGTTTACAC	TGCTATAAAA	AAATTATTAC	CCAAGCAGAG	ATATCCCTTA	4550
AGCCTGGAGG	AATAATAATT	TTAGAACATG	GAGCAACACA	AGCAGAAAGCT	4600
ATCTTATTGT	TGTTAAAAAA	CAACATATGG	ACAAATGTAA	TAAGTCATAC	4650
TGATCTTACA	AATAAAAATC	GTTTATTAC	AGCATATAAG	TATAAAATAT	4700
AACTTAATTA	TGTTGkagAa	AAAACAAAAA	ATAAAAATAA	GATATTAAaT	4750
ATTTtttttA	aTAAAATTAA	GCAatTACTA	ATATCTTTT	TTGGGrTCGtt	4800
yaTtGsATwA	GAAACTTTGG	rGGrTrrCTa	TGAACAAACA	ACCATnCAAC	4850
GGCCAAnTAC	ATnnCAGGnT	TGGGGTCATA	GGGGCCACGC	TTTATGTACG	4900
TACAACCCn	ACTGAAATTc	TGGnTTGnTT	TGGGGGGnAA	nTGGGTATCG	4950
CAACnCTnTC	CCCCCCCCCT	GG			4972

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 569 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 209..569

- 51 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

- 52 -

ACA CGA GCA GGG AAT TTT CGT TTT ACT CAA GAT GGT TTT TTA AAT GAT C 569  
Thr Arg Ala Gly Asn Phe Arg Phe Thr Gln Asp Gly Phe Leu Asn Asp  
105 110 115 120

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 123 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ser Leu Phe Ile Xaa Ala Asn Arg Tyr Glu Asn Pro \* Xaa Arg Xaa  
1 5 10 15

Gly Thr Val Ser Asn Asn Ile Ala Asn Ala Asn Thr Ile Gly Tyr Lys  
20 25 30

Gln Gln Gln Val Val Phe Gln Asp Leu Phe Ser Gln Asp Leu Ala Ile  
35 40 45

Gly Phe Thr Gly Ser Gln Gly Pro Asn Gln Ala Gly Met Gly Ala Gln  
50 55 60

Val Gly Ser Val Arg Thr Ile Phe Thr Gln Gly Ala Phe Glu Pro Gly  
65 70 75 80

Asn Ser Val Thr Asp Pro Ala Ile Gly Gly Lys Gly Phe Phe Gln Val  
85 90 95

Thr Leu Glu Asp Lys Val His Tyr Thr Arg Ala Gly Asn Phe Arg Phe  
100 105 110

Thr Gln Asp Gly Phe Leu Asn Asp

- 53 -

115

120

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1450 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..414

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1083..1450

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GA TCT AAA GAG TCT ACA TAT ATT GCC CGA ATT GAA AAT TCT ACA AGT  
Ser Lys Glu Ser Thr Tyr Ile Ala Arg Ile Glu Asn Ser Thr Ser  
1 5 10 15

47

GAA AAA ACA CTA AAT GAT CTT GAT ATA CTT TTA AAA GAT GTG ATG TTA  
Glu Lys Thr Leu Asn Asp Leu Asp Ile Leu Leu Lys Asp Val Met Leu  
20 25 30

95

ACA TCA AAA AAG CAT GAA TCA CGT AGA CTT GCA GAG TCT GTA CAT CAA  
Thr Ser Lys Lys His Glu Ser Arg Arg Leu Ala Glu Ser Val His Gln  
35 40 45

143

AAT ATT CTT ACC CAC CTT ATA CAA AAA AAT TAT AAT ACT CAC AAT GGT

191

- 54 -

Asn Ile Leu Thr His Leu Ile Gln Lys Asn Tyr Asn Thr His Asn Gly

50 55 60

GGG ATA AAA TCT GCA CCT TTT CAT GTT CTT ATA GGA CCC AAA ATA CCA 239  
Gly Ile Lys Ser Ala Pro Phe His Val Leu Ile Gly Pro Lys Ile Pro  
65 70 75

AGT ATT CTT GTT GAA GTA GGT TAC TGT AGT AAT AAA GCT GAA GCA CAG 287  
Ser Ile Leu Val Glu Val Gly Tyr Cys Ser Asn Lys Ala Glu Ala Gln  
80 85 90 95

CGT CTG GCA TCT AGT AAT TAC CAA AAA GCA TTA ATA GAA GGA TTA GCT 335  
Arg Leu Ala Ser Ser Asn Tyr Gln Lys Ala Leu Ile Glu Gly Leu Ala  
100 105 110

AAA GGT ATT TTC TGT TAC CTA AAA AAA CTA CAT CAC CTT GAT ATT TAC 383  
Lys Gly Ile Phe Cys Tyr Leu Lys Lys Leu His His Leu Asp Ile Tyr  
115 120 125

TCT AGT TTT ATY CTA TCT AAT TGC ACT TAA T AGCTTGGACA ATTATTATAT 434  
Ser Ser Phe Ile Leu Ser Asn Cys Thr \*  
130 135

GAAGGGTATC CATGTGAAGG TACCTGGTTA AGCTTTAAA TGAAAAATT ATGCAACCAT 494

ACYTTATTCC TTCAGAGGAG CTTCATTATG AAAGTAAAAA CTCTTCCAT GGCTATTTA 554

GCTTGTTAT TAGTAGCTAA CAGTGCATT TCGGCTGACT TCCCTATTGG TGTCTTTAAT 614

TCTCAATCCA TTGCCATGGA GAGTGAAGCA GCTAAGGCCG CTCAAAAAAA ATTACAATCA 674

GAATTTGGTA ATGAAAAAAC ACAACTTGAA AACAGCAAA AGWTTGCMMA CAAAAGCTGA 734

TGATTTACAA GCTWAGTCAG CAGCTATGTY TAACCAAGCA CGTGAAGATA AACAAAGAGA 794

ATTTCTTGAA CTTCGTCGTA ATTTCGAAGA AAAATYTCGT GACTTGCAA TACGTGTCGA 854

- 55 -

ACAAGCTGAA AACACATTAC GTCAATATNT AGCTGAACAA ATNTATNTTG CTGCTGAAAC	914		
TATAGCAAAA AAGAAAGGGT TAAACTTGT TTGATAGTGT TAGGGAAGTG TAATGTACCT	974		
TGAAAAAAAT TTAGATATTA CAAAGAAATT YTTGAAGCCA TAAATGCTGC ATGGAAAAAA	1034		
GGTGGAAAGTA AACTTCCAGA GATGGCAAAC CGGAAAAAT AACAG ATG CCC CAG TAT	1091		
	Met Pro Gln Tyr 1		
AAA CTT TCA GAA ATT GCT AAA CTT TTA AAC TTA ACA TTA CAA GGT GAT	1139		
Lys Leu Ser Glu Ile Ala Lys Leu Leu Asn Leu Thr Leu Gln Gly Asp			
5	10	15	20
GAT ATT GAA GTT GTA GGC GTA AAT ACA CTT CAA GAT GCA TCA CCA AAT	1187		
Asp Ile Glu Val Val Gly Val Asn Thr Leu Gln Asp Ala Ser Pro Asn			
25	30	35	
GAG ATA AGT TTT CTA GCA AAT GCT AAA TAT ATT CAC CAG CTT GTT TTG	1235		
Glu Ile Ser Phe Leu Ala Asn Ala Lys Tyr Ile His Gln Leu Val Leu			
40	45	50	
TCA CAG GCT GGT GCT ATT ATT CTT TCA AAA GAA TAT GCT AGT CGT GTT	1283		
Ser Gln Ala Gly Ala Ile Ile Leu Ser Lys Glu Tyr Ala Ser Arg Val			
55	60	65	
CCA CGA GCA CTA ATC AGT ACT GAA CCA TAT AGA GAT TTT GGT AGA GTT	1331		
Pro Arg Ala Leu Ile Ser Thr Glu Pro Tyr Arg Asp Phe Gly Arg Val			
70	75	80	
CTT TCT TTA TTC TCT ATA CCT CAA GGA TGT TTT GAT GGT ATA AGT CAT	1379		
Leu Ser Leu Phe Ser Ile Pro Gln Gly Cys Phe Asp Gly Ile Ser His			
85	90	95	100
CAA GCT TAT ATA CAC CCT ACA GCA CAA GTC TCT AAA ACA GCT ACT ATC	1427		
Gln Ala Tyr Ile His Pro Thr Ala Gln Val Ser Lys Thr Ala Thr Ile			
105	110	115	

- 56 -

TAT CCT TIn GTT TTT ATA GGA TC

1450

Tyr Pro Xaa Val Phe Ile Gly

120

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 137 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ser Lys Glu Ser Thr Tyr Ile Ala Arg Ile Glu Asn Ser Thr Ser Glu

1 5 10 15

Lys Thr Leu Asn Asp Leu Asp Ile Leu Leu Lys Asp Val Met Leu Thr

20 25 30

Ser Lys Lys His Glu Ser Arg Arg Leu Ala Glu Ser Val His Gln Asn

35 40 45

Ile Leu Thr His Leu Ile Gln Lys Asn Tyr Asn Thr His Asn Gly Gly

50 55 60

Ile Lys Ser Ala Pro Phe His Val Leu Ile Gly Pro Lys Ile Pro Ser

65 70 75 80

Ile Leu Val Glu Val Gly Tyr Cys Ser Asn Lys Ala Glu Ala Gln Arg

85 90 95

Leu Ala Ser Ser Asn Tyr Gln Lys Ala Leu Ile Glu Gly Leu Ala Lys

100 105 110

Gly Ile Phe Cys Tyr Leu Lys Lys Leu His His Leu Asp Ile Tyr Ser

- 57 -

115

120

125

Ser Phe Ile Leu Ser Asn Cys Thr \*

130

135

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 123 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10: —

Pro Gln Tyr Lys Leu Ser Glu Ile Ala Lys Leu Leu Asn Leu Thr Leu

1

5

10

15

Gln Gly Asp Asp Ile Glu Val Val Gly Val Asn Thr Leu Gln Asp Ala

20

25

30

Ser Pro Asn Glu Ile Ser Phe Leu Ala Asn Ala Lys Tyr Ile His Gln

35

40

45

Leu Val Leu Ser Gln Ala Gly Ala Ile Ile Leu Ser Lys Glu Tyr Ala

50

55

60

Ser Arg Val Pro Arg Ala Leu Ile Ser Thr Glu Pro Tyr Arg Asp Phe

65

70

75

80

Gly Arg Val Leu Ser Leu Phe Ser Ile Pro Gln Gly Cys Phe Asp Gly

85

90

95

Ile Ser His Gln Ala Tyr Ile His Pro Thr Ala Gln Val Ser Lys Thr

- 58 -

100

105

110

Ala Thr Ile Tyr Pro \* Val Phe Ile Gly

115

120

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 559 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..557

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GA TCA AAG CCG CAT TTA CNG CAA GAG TTA GAA ATT GAA GTT TTG AAA 47

Ser Lys Pro His Leu Xaa Gln Glu Leu Glu Ile Glu Val Leu Lys

1

5

10

15

AAA GAA GAC TTT GGG CGT CAT ATT GTT AAA TTA TGC TGG AAA GGT TCT 95

Lys Glu Asp Phe Gly Arg His Ile Val Lys Leu Cys Trp Lys Gly Ser

20

25

30

TTA TCA AAT ATC TTT TTT TCC TAT GGG GAT ATC CCG CAC CCA CCT TAT 143

Leu Ser Asn Ile Phe Phe Ser Tyr Gly Asp Ile Pro His Pro Pro Tyr

35

40

45

ATA CAT CAA AGT AAT AAG GTT CAG GAT AAG GAA AGA TAT CNT ACN GTA 191

- 59 -

Ile His Gln Ser Asn Lys Val Gln Asp Lys Glu Arg Tyr Xaa Xaa Val			
50	55	60	
TAC TCT ATA TTA CAT AAN CTG GGT TCT GTA GCA GCT CCT ACA GCT GGA			239
Tyr Ser Ile Leu His Xaa Leu Gly Ser Val Ala Ala Pro Thr Ala Gly			
65	70	75	
TTA CNC TTT TCT GAA ACT AGC CGT NAT AAA TTA CAC AAA NAT GGT ATT			287
Leu Xaa Phe Ser Glu Thr Ser Arg Xaa Lys Leu His Lys Xaa Gly Ile			
80	85	90	95
AGT TGG GCA TAA ATC CCT CTT CAC GTG GGA TAT GGA ACA TTC AGT CCC			335
Ser Trp Ala * Ile Pro Leu His Val Gly Tyr Gly Thr Phe Ser Pro			
100	105	110	
GTC CTC TGC AAT GAC ATC CCA AAA CAT CTT ATC CNT TCT GAG TTT GTT			383
Val Leu Cys Asn Asp Ile Pro Lys His Leu Ile Xaa Ser Glu Phe Val			
115	120	125	
CAC TTT CCT GAA ACT ACN TTT TCC ACT ATA TTA AAT GCA CGG TTT GCA			431
His Phe Pro Glu Thr Xaa Phe Ser Thr Ile Leu Asn Ala Arg Phe Ala			
130	135	140	
NGG GAA TAC CTA TGT TCT GCC ATA GGG GAC CCA CTG TTG TCC CCA CCA			479
Xaa Glu Tyr Leu Cys Ser Ala Ile Gly Asp Pro Leu Leu Ser Pro Pro			
145	150	155	
TTG GAN GGG TGT TAT CTT ACC CCT TTC GCC CGG GGT TCC CCT CCC CAA			527
Leu Xaa Gly Cys Tyr Leu Thr Pro Phe Ala Arg Gly Ser Pro Pro Gln			
160	165	170	175
CCC TAT TCC ATT GNG TTT TCC TCT CAA ATT AT			559
Pro Tyr Ser Ile Xaa Phe Ser Ser Gln Ile			
180	185		

- 60 -

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 185 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ser Lys Pro His Leu Xaa Gln Glu Leu Glu Ile Glu Val Leu Lys Lys  
1 5 10 15

Glu Asp Phe Gly Arg His Ile Val Lys Leu Cys Trp Lys Gly Ser Leu  
20 25 30

Ser Asn Ile Phe Phe Ser Tyr Gly Asp Ile Pro His Pro Pro Tyr Ile  
35 40 45

His Gln Ser Asn Lys Val Gln Asp Lys Glu Arg Tyr Xaa Xaa Val Tyr  
50 55 60

Ser Ile Leu His Xaa Leu Gly Ser Val Ala Ala Pro Thr Ala Gly Leu  
65 70 75 80

Xaa Phe Ser Glu Thr Ser Arg Xaa Lys Leu His Lys Xaa Gly Ile Ser  
85 90 95

Trp Ala \* Ile Pro Leu His Val Gly Tyr Gly Thr Phe Ser Pro Val  
100 105 110

Leu Cys Asn Asp Ile Pro Lys His Leu Ile Xaa Ser Glu Phe Val His  
115 120 125

Phe Pro Glu Thr Xaa Phe Ser Thr Ile Leu Asn Ala Arg Phe Ala Xaa  
130 135 140

- 61 -

Glu Tyr Leu Cys Ser Ala Ile Gly Asp Pro Leu Leu Ser Pro Pro Leu  
 145                    150                    155                    160

Xaa Gly Cys Tyr Leu Thr Pro Phe Ala Arg Gly Ser Pro Pro Gln Pro  
 165                    170                    175

Tyr Ser Ile Xaa Phe Ser Ser Gln Ile  
 180                    185

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 477 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2..294

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

T ATA AAA CAT TAG CGN CTT TNG TAT TTG GAC TTC AAA AAA ATT TTT	46
Ile Lys His * * Leu * Tyr Leu Asp Phe Lys Lys Ile Phe	
1                    5                    10                    15	

AAT TAT ATA GGA GAA CAT TCA CCA TTA AAA CGT AAT GTC ANT ATG GAA	94
Asn Tyr Ile Gly Glu His Ser Pro Leu Lys Arg Asn Val * Met Glu	
20                    25                    30	

GAT GTA GGT AAA TCT GCT GTT TTT TTA GCT TCA GAC CTN TCA TCA GGA	142
Asp Val Gly Lys Ser Ala Val Phe Leu Ala Ser Asp * Ser Ser Gly	

- 62 -

35

40

45

TTA ACC ATA CAT GCT TTA TAC AAC ATA TTG TGA GTT ACA ATA GCC ATA 238  
 Leu Thr Ile His Ala Leu Tyr Asn Ile Leu \* Val Thr Ile Ala Ile  
 65 70 75

ACA CAT TTA TAT TCT ATA TAA TAA CAG TAG AAT AAT AAT AGA ATA TTT      286  
 Thr His Leu Tyr Ser Ile \*    Gln \*    Asn Asn Asn Arg Ile Phe  
                               80                        85                        90                        95

TTT ATG ACC ATTTGTATCT ATACAATAGT AAATAGATTA ATACATATAA GACTATATT C 344  
Phe Met Thr

TTTTGAGAG CAACTTAAAG GAGCGGTTAT GGCTTTAGTT ACAAAAAGAAG AAGTACTTCA 404

**ATACCATAGT** GAACCCCCGAC CAGGTAAACT TGAAGTATT TCTATAAAAAC CATGTAAAC 161

ACAAAAAAAGAT CC

(2) INFORMATION FOR SEO ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 97 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Ile Lys His \* Xaa Leu Xaa Tyr Leu Asp Phe Lys Lys Ile Phe Asn

- 63 -

1

5

10

15

Tyr Ile Gly Glu His Ser Pro Leu Lys Arg Asn Val Xaa Met Glu Asp  
20 25 30

Val Gly Lys Ser Ala Val Phe Leu Ala Ser Asp Xaa Ser Ser Gly Val  
35 40 45

Thr Gly Glu Xaa Phe Leu Leu Met Leu Xaa Gln \* Phe Arg Tyr Leu  
50 55 60

Thr Ile His Ala Leu Tyr Asn Ile Leu \* Val Thr Ile Ala Ile Thr  
65 70 75 80

His Leu Tyr Ser Ile \* \* Gln \* Asn Asn Asn Arg Ile Phe Phe  
85 90 95

Met

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 525 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2..525

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

- 64 -

G GAA TTG TTA GTA TTC TCC CAG AAC AGA AGC CAA AAT ATT TGG CTA	46
Glu Leu Leu Val Phe Ser Gln Asn Arg Ser Gln Asn Ile Trp Leu	
1 5 10 15	
 CTT ACA TTA CCT ATT TTT GTG TTA GGT ATA GCA CAA GGT ATA TCA TTT	94
Leu Thr Leu Pro Ile Phe Val Leu Gly Ile Ala Gln Gly Ile Ser Phe	
20 25 30	
 CCT TTA GTA AAC AGC CAC ATT ACA TCA CTT GCA CCA ACA TCC AAC AGA	142
Pro Leu Val Asn Ser His Ile Thr Ser Leu Ala Pro Thr Ser Asn Arg	
35 40 45	
 GCT ATT GTT ATG GCT ATA AAC AGT ACA TTT ATG AGG TTA AGT CAG AGT	190
Ala Ile Val Met Ala Ile Asn Ser Thr Phe Met Arg Leu Ser Gln Ser	
50 55 60	
 ATT TCG CAA ATG GTT TTT GGT ATT GGA TGG TCA TTT TTT GGT TGG CCT	238
Ile Ser Gln Met Val Phe Gly Ile Gly Trp Ser Phe Phe Gly Trp Pro	
65 70 75	
 GGT CCT TTT ATA TTT GGT CTT TTT ACT TCT ATT ATA TTA GCC CTC TTA	286
Gly Pro Phe Ile Phe Gly Leu Phe Thr Ser Ile Ile Leu Ala Leu Leu	
80 85 90 95	
 ATT ATG AAG TAT TTT CAA GAT GTA ACC CAA TAT CAC CTA TTT TTG ATA	334
Ile Met Lys Tyr Phe Gln Asp Val Thr Gln Tyr His Leu Phe Leu Ile	
100 105 110	
 AGT AGT AAA TTT TAT TAT TAA AAA GCT TAG TTA GTT AAG ATT ACA TAT	382
Ser Ser Lys Phe Tyr Tyr * Lys Ala * Leu Val Lys Ile Thr Tyr	
115 120 125	
 ATT ATA TAC AAT TAC TAT AAC ATT AAC TAA TTA CTA ACT ATT ACT TCC	430
Ile Ile Tyr Asn Tyr Tyr Asn Ile Asn * Leu Leu Thr Ile Thr Ser	
130 135 140	
 AAT TGA TTA ATT GAT GCT ATT TAA AGA GGA TAT ATT AAT GAT GTC ATG	478

- 65 -

Asn \* Leu Ile Asp Ala Ile \* Arg Gly Tyr Ile Asn Asp Val Met  
145 150 155

GCT CAC AAT AGG TGT TAT CCT TGG ATT AGT GCA TGG GAT CCA GGT CC 525  
Ala His Asn Arg Cys Tyr Pro Trp Ile Ser Ala Trp Asp Pro Gly  
160 165 170

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 174 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Glu Leu Leu Val Phe Ser Gln Asn Arg Ser Gln Asn Ile Trp Leu Leu  
1 5 10 15

Thr Leu Pro Ile Phe Val Leu Gly Ile Ala Gln Gly Ile Ser Phe Pro  
20 25 30

Leu Val Asn Ser His Ile Thr Ser Leu Ala Pro Thr Ser Asn Arg Ala  
35 40 45

Ile Val Met Ala Ile Asn Ser Thr Phe Met Arg Leu Ser Gln Ser Ile  
50 55 60

Ser Gln Met Val Phe Gly Ile Gly Trp Ser Phe Phe Gly Trp Pro Gly  
65 70 75 80

Pro Phe Ile Phe Gly Leu Phe Thr Ser Ile Ile Leu Ala Leu Leu Ile  
85 90 95

- 66 -

Met Lys Tyr Phe Gln Asp Val Thr Gln Tyr His Leu Phe Leu Ile Ser

100

105

110

Ser Lys Phe Tyr Tyr \* Lys Ala \* Leu Val Lys Ile Thr Tyr Ile

115

120

125

Ile Tyr Asn Tyr Tyr Asn Ile Asn \* Leu Leu Thr Ile Thr Ser Asn

130

135

140

\* Leu Ile Asp Ala Ile \* Arg Gly Tyr Ile Asn Asp Val Met Ala

145

150

155

160

His Asn Arg Cys Tyr Pro Trp Ile Ser Ala Trp Asp Pro Gly

165

170

**(2) INFORMATION FOR SEQ ID NO:17:**

**(i) SEQUENCE CHARACTERISTICS:**

- (A) LENGTH: 846 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

**(ii) MOLECULE TYPE: DNA**

**(iii) SEQUENCE DESCRIPTION: SEQ ID NO:17:**

TATTTACTCG CGCGGCCGGG CGTCTTACAC AAATGGATCC CTTGCANTAA TCCAAGGATA	60
ACNCCTATTG TGANCCATGA ACATCATCAN NATATCCTCT TTANATAGCA TCNANNNNTC	120
AANNGGAATT AACAGTTACT ANNTAGTTAA TGTCATAGTA ATTGTCNATA ATATATGTAA	180
TCTTAACTAA CTAAGCTNNT TAATAATAAA ATTNACTACT TATCAAANAAT AGGTGATATN	240
GGGTTACATC TTGAAAATAC TTNCCTAAAT TANGAGGGCT AATATAATNG AANTAAAAAG	300
ACCANATATA AAAGGACCAG GCCAACCAAA AAATGACCAT CCAATACCNA AAACAATTGG	360

- 67 -

CGAAAATACT CTGACTTAAC CTCANAAATG TACTGTTTAT AGCCATATCA ATAGCTCTGT	420
TGGATGTNGG NGCAATTGAT GTAATGTGGC TGTNTACTAN ANGAAATGAT NTACCTCGTG	480
CTATNCCTAN NACAANAATA NGTAATGTAA GTANCCNAAT ATCTTGGCTT TGTAATGGGA	540
GAATAATNNC AAGTCCTTGG GAAATNAANT TACNNCCAGC CAGCTATNNT AAGCAGTTCT	600
NTGGTGACTA TACGTCTTAC TNAANTCGTG CCAAAGATTAA AATANNCGAT AATCGCNCTN	660
CCTAAANCAN GCAATACTAA AATGGTTTCT NCCTANCTTG GNATANGGTG GAAGCNCGGA	720
CAGAATTNNAN TTCGCNANTT TANANNGGAA NATNCGTNAA NTTANTCGGG GCCCANNCCN	780
AAATTCCCTNA NTCNATANAN NAACTNNCTN CTNTAAAANG GCCNACTGGA NTNGTTAAAT	840
GAAATA	846

## (2) INFORMATION FOR SEQ ID NO:18:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 855 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (iii) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GATTNTTAT CGATCACTNT AGACGCGATT TGGGNAACAC TTACCTGGTA NCCACCCGGG	60
TGGAAAAATC GATGGGCCCG CGGCCGCTCT AGAAGTACTC TCGAGAAGCT TTTTGAATT	120
TTTGGATCCT CAACACAGGG TATGGATTAA AACAACTTTA GCTCTAACAG GAGCATT	180
TAATATATTC CCTGGTAGAA CAATATCTAC TCAAGAAAAT CTGTCTATTG GTTTTCAACT	240
AAAAAAAACT TTTAACCTT TTCATTGGAC CATCTTACTC TTAGATGAAC ATTATATGTC	300
TTCGCCAAGA ATTGCAGCAG CAATTATGCC TGCACAGCTT GCTGGAGTTA AAAACATTAT	360

- 68 -

AGCTGTTGG ACCAGTAAAA ATAACCGACT GACCGCTGAA AAAATCTCAC CTGCTTTACT	420
AACAACATTA GAACTTCAG GAGTTAACAT AGCCCTAACCA CTTACCCACA CTGAAACTGA	480
ACTTCTTATT CATCAATTAA TGAAAATAGG TATTGGAAAC CTGTTATATT TTTTAAAAGA	540
AGAAGACATA CTACATATAT CTACTATACC TGTACTACCT TTCTGGAAAG AATATACTTC	600
TCATCGACTT GTTATAGAAA AAGATGCTGG CNTTAATACA GAAATCCTCC AATGGGCNCA	660
TCCTCATTCA ATTATTGAAC AAATAGCAAC AGAACCATAC TCTGAAANAT ATCCCAGATG	720
CACTTTACTG TGCTAGCTCA TCCANTAAAA ACTATNCTCA TANAGNATCC CCAGAATTTT	780
TCATNATGGA CTTGAACCTA TTTGGATTCA NCCCAACNCT TCCTCCAANC CTCCTTCTC	840
CATACACCAT GGGGA	855

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1082 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TATCTNGTTG ANTCAATAAA ACTTTTGCCCCNTNAAN TTTCATNANN AAAAAAAACAA	60
NATTNCTGGG GGNCCCNCTCC CAAAAAANNC AATCANTNNNG AANCTTGNCCT CTTTATTNNNG	120
NTTTTNANAC TATAATATNT NTTATCNATA ATNNATCNNT ATACTNATTCTNATTCACT	180
NACANNGGNAGNAANNTTA ATCTNAAANA CTNCNAAGGG GGNNNTNATA NTNTTTNTTT	240
NTTTNTCCCN TNNAATNNAT AACCNCCAC CCNNATTANT TNNAATNNAT ACCATANCNN	300
CCTTTCAAAC TGTACACATA NTANNNAAANN ACACTCNANC NTTTNCATC CTCTCTANTN	360

- 69 -

CCNACTCCNA TNNANCTNTT CCCCCATNCC TATNTNTCNC TGCTTCCCAG NTTNNACNTN	420
NCTTNNTTTC ACANTATTCC TATCCAANCT AACATNTNTN NTNTCNTNCT CCTTNTNTNT	480
TATNTNTTTC TNNTACCTNN CACTGACANT CTATNANTNA NNTCNNATAAC TNNTATANCT	540
NTANGCNANT NTATCTANAA NTNTANCNNN NNATCNTNAC NGCCGTNNAT NTNNNNNCAN	600
TTANNTANNN CTANCNTNNC CAANNNCNTA TNTATNAATA ACNACTATCC NATATTNNAT	660
TNNNTNNNTNT CNTANNCAAA TNATTTANGC NCACNNCACT ANGTNATATN ANNATTNTAT	720
ATTNTGAANC TTCTNGGCTT CNCNAATANT ACCANTNNNC ANCNTCNNT NCATCTNNNT	780
NTACTTCNTA CCATANCGCT CTCNAGNNTC ACTACTTCTA NTAGTNATCN TCTACTGCCN	840
ATGGCNNNNN GCNNNNCGAN AGNTATNCAC NTACANTNNC NTCTACTATN TANATCTANN	900
NCNTCCGNNG CCTNCNGTAC GNNTNGGCNA ANTGNNTAC TTTNCNTNTA TCTAGTCNCA	960
TCAGNNNTNG ANTCCCTCAAN CNNGCTCTAN TTACATGTNN NNTNATGCNC TANANCGNNA	1020
CNTCTATCCT TCNANTCTGC NCTNANTNTA TANACTCTNN NNNATCNNCN AANCTATNTC	1080
CC	1082

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 354 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:20

CTCCCNTNNC NCTAAAGTGGAA NTGGCGCGCT GCAGGGTCGAC ACTAGTGGAT CTTGATATAC	60
TTTTAAAAGA TGTGATGTTA ACATCAAAAA AGCATGAATC ACGTTAGACT TGCAGAGTCT	120
GTACATCAAA ATATTCTTTA CCCACCTTAA TACGAAAANA AATNNTTATN CNCCNCNATG	180
GGTGGGGNTN AAATCCTNGC CCCNTTNCCT TGTCNTTTA GGGAAACCCCC NAATTCCCCN	240
NGTTATTCCCT CTGTTTGAAA NTTCTGGTTN CCCGGCCCTN TNACCAANAG CTTGANNNCC	300
NCCTCGTCTT GGGGCATCCT CNTGTTTATT TTCCCTCNAN CNCCCCCTTN ACTN	354

- 70 -

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 477 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:21

GGATCTTTT GTGTTTACA TGGTTTATA GGAAATACTT CAAGTTACC TGGTCGGGGT	60
TCACTATGGT ATTGAAGTAC TTCTTCTTT GTNACTAAAG CCATAACCGC TCCTTTAAGT	120
TGTTCTCAA AAGAATATAG TCTTATATGT ATTAATCTAT TTACTATTGT ATAGATACAA	180
TAGGTCTAAA AAAATATTCT ATTATTATTC TACTGTTATT ATATAGAATA TAAATGTGTT	240
ATGGCTATTG TAACTCACAA TATGTTGTAT AAAGCATGTA TGGTTAAATA CCTAAATTAT	300
TGTNCCAGCA TCAACAAAAA NAATTCACCG GTTACTCCTG ATGANAGGTC TGAAGCTAAA	360
AAAACAGCAG ATTTACCTAC ATCTTCCATA NTTACATTAC GTTTTAATGG TGAATGTTCT	420
CCTATATAAT TAAAAATTTT TTTGAAGTCC AAATACNAAA GNCGCTAATG TTTTATA	477

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 568 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:22

- 71 -

GATCATTAA AAAACCACATCT TGAGTAAAAC GAAAATTCCC TGCTCGTGTA TAGTGTACTT	60
TATCCTCTAA TGTAACCTGA AAAAAACCTT TTCCACCAAT AGCAAGATCT GTTACACTAT	120
TGCCAGGGTTC AAAAGCACCC TGTGTAAAAA TTGTGCGAAC ACTTCCAACC TGTGCTCCC	180
TACCAGCCTG GTTGGCCCC TGACTTCCAG TAAAACCTAT TGCTAAATCT TGACTAAACA	240
GGTCTTGAAA CACTACCTGT TGCTGCTTAT ACCCAATGGT ATTTGCGTTA GCAATATTAT	300
TGGAGACAGT ACCANCCCTG TNCTATGGGT TTTCATACCT GTTGGCANCA ATAAACAAAC	360
TCCCCATCAT GATAACATCT CCTAAAAAAT AATTTCATGG NGGNAAAAT GTTACCTACA	420
CATCTCTATT TTNAAGCAA AAAACCCATG CCCAANAAAA TTTTTGGGCC NAATTAATAT	480
ACTTAATCTA ATAAACTTTT TTGGGTAATN AAAAAAAATT AATTTTTAA ACTTGGTTN	540
ACCAACCTTT TCTCCTTACT TTTTAACC	

- 72 -

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 477 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:23

GGTACCCCAC CCGGGTGGAA AATCGATGGG CCCGCAGCCG CTCTAAAANT	50
ACTCTCGAGA AGCTTTTGA ATTCTTTGA TCCCCAGGAA TAACTTGTG	100
ACGGAATTT ACATTTCTA TCCCTGAAA TAAAAAAACT TTACCTTGTA	150
GTTCAATTAGGAAAGAT TGGAGTACTG TGATTCCACC TGATTGCGCC	200
ATAGCTTCTA AAATTAGAAC TCCAGGCATG ACAGGAAATC CAGGGAAAT	250
GACCCNGAAA AAATGGTCA TTAATACTAA CATTTTTATA AGCTTTAATA	300
TATTTGCCAG CATTAAATTC AATAACTCTA TCTACAATTA AAAAGGGATA	350
ACGGTGGGGA ATTTACTGTA AAATTCTTG GATATTTGG AGGTATGGAT	400
GGGGACATTA ATTTCTAT ATATATGCTC TTTTCTTT CNAAAATTT	450
TCAGCTTTT TATCCCNTAA AAACCTC	467

**CLAIMS:**

1. A vaccine composition for the prophylaxis or treatment of infection in an animal or bird by *Lawsonia intracellularis* or related microorganism, said vaccine composition comprising an immunogenic, non-pathogenic form of *L. intracellularis* or related microorganism or an immunogenic component thereof and one or more carriers, diluents and/or adjuvants suitable for veterinary or pharmaceutical use.
2. A vaccine composition according to claim 1 wherein the composition is for the prophylaxis or treatment of infection in pigs by *L. intracellularis* or related microorganism.
3. A vaccine composition according to claim 2 wherein the non-pathogenic form of *L. intracellularis* or related microorganism is an attenuated strain of the microorganism.
4. A vaccine composition according to claim 2 wherein the non-pathogenic form of *L. intracellularis* or related microorganism is a killed preparation of the microorganism.
5. A vaccine composition according to claim 4 wherein the non-pathogenic form of *L. intracellularis* is a formalin-killed preparation of the microorganism.
6. A vaccine composition according to claim 1 or 2 wherein said composition comprises a peptide, polypeptide, protein, carbohydrate, lipid or nucleic acid molecule or a combination thereof from *L. intracellularis* or related microorganism in an amount effective to induce a protective immune response agent *L. intracellularis* or related microorganism.
7. A vaccine composition according to claim 6 wherein the composition comprises a peptide, polypeptide, protein or a derivative thereof from *L. intracellularis* or related microorganism.

- 74 -

8. A vaccine composition according to claim 7 wherein the peptide, polypeptide or protein is in recombinant form.
9. A vaccine composition according to claim 7 or 8 wherein the composition comprises a refolding/heatshock protein, a flagellar basal body rod protein, S-adenosylmethionine:tRNA ribosyltransferase-isomerase, autolysin, enoyl-(acyl-carrier-protein) reductase or a glucarate transporter or derivative thereof.
10. A vaccine composition according to claim 9 wherein the protein is GroEL having an amino acid sequence set forth in SEQ ID NO:2 or is a protein having at least about 40% similarity thereto.
11. A vaccine composition according to claim 9 wherein the protein is GroES having an amino acid sequence set forth in SEQ ID NO:4 or is a protein having at least about 40% similarity thereto.
12. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:1 or a sequence having at least about 40% similarity thereto.
13. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:3 or a sequence having at least about 40% similarity thereto.
14. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:5 or a sequence having at least about 40% similarity thereto.
15. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:6

- 75 -

or a sequence having at least about 40% similarity thereto.

16. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:8 or a sequence having at least about 40% similarity thereto.

17. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:11 or a sequence having at least about 40% similarity thereto.

18. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:13 or a sequence having at least about 40% similarity thereto.

19. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:15 or a sequence having at least about 40% similarity thereto.

20. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:17 or a sequence having at least about 40% similarity thereto.

21. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:18 or a sequence having at least about 40% similarity thereto.

22. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:19 or a sequence having at least about 40% similarity thereto.

23. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:20 or a sequence having at least about 40% similarity thereto.
24. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:21 or a sequence having at least about 40% similarity thereto.
25. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:22 or a sequence having at least about 40% similarity thereto.
26. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein having an amino acid sequence of SEQ ID NO:7 or a sequence having at least 40% similarity.
27. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein having an amino acid sequence of SEQ ID NO:9 or a sequence having at least 40% similarity.
28. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein having an amino acid sequence of SEQ ID NO:10 or a sequence having at least 40% similarity.
29. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein having an amino acid sequence of SEQ ID NO:12 or a sequence having at least 40% similarity.
30. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein having an amino acid sequence of SEQ ID NO:14 or a

- 77 -

sequence having at least 40% similarity.

31. A vaccine composition according to claim 8 wherein the composition comprises a peptide, polypeptide or protein having an amino acid sequence of SEQ ID NO:16 or a sequence having at least 40% similarity.

32. A method for vaccinating an animal or bird against infection by *L. intracellularis* or related microorganism or treating an animal or bird infected by *L. intracellularis*, said method comprising administering to said animal or bird an effective amount of a non-pathogenic form of *L. intracellularis* or related microorganism or an immunogenic component thereof for a time and under conditions sufficient to induce a protective immune response against *L. intracellularis* or related microorganism.

33. A method according to claim 32 wherein the animal is a pig.

34. A method according to claim 33 wherein the non-pathogenic form of *L. intracellularis* or related microorganism is an attenuated strain of the microorganism.

35. A method according to claim 33 wherein the non-pathogenic form of *L. intracellularis* or related microorganism is a killed preparation of the microorganism.

36. A method according to claim 35 wherein the non-pathogenic form of *L. intracellularis* is a formalin-killed preparation of the microorganism.

37. A method according to claim 32 and 33 wherein said immunogenic component comprises a peptide, polypeptide, protein, carbohydrate, lipid or nucleic acid molecule or a combination thereof from *L. intracellularis* or related microorganism in an amount effective to induce a protective immune response against *L. intracellularis* or related microorganism.

38. A method according to claim 37 wherein said immunogenic component comprises a

peptide, polypeptide, protein or a derivative thereof from *L. intracellularis* or related microorganism.

39. A method according to claim 38 wherein the peptide, polypeptide or protein is in recombinant form.

40. A method according to claim 29 or 30 wherein the immunogenic component is a refolding/heatshock protein, a flagellar basal body rod protein, S-adenosylmethionine: tRNA ribosyltransferase-isomerase, autolysin, enoyl-(acyl-carrier-protein) reductase or a glucarate transporter or derivative thereof.

41. A method according to claim 40 wherein the protein is GroEL having an amino acid sequence set forth in SEQ ID NO:2 or is a protein having at least about 40% similarity thereto.

42. A method according to claim 40 wherein the protein is GroES having an amino acid sequence set forth in SEQ ID NO:4 or is a protein having at least about 40% similarity thereto.

43. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:1 or a sequence having at least about 40% similarity thereto.

44. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:3 or a sequence having at least about 40% similarity thereto.

45. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:5 or a sequence having at least about 40% similarity thereto.

- 79 -

46. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:6 or a sequence having at least about 40% similarity thereto.
47. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:8 or a sequence having at least about 40% similarity thereto.
48. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:11 or a sequence having at least about 40% similarity thereto.
49. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:13 or a sequence having at least about 40% similarity thereto.
50. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:15 or a sequence having at least about 40% similarity thereto.
51. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:17 or a sequence having at least about 40% similarity thereto.
52. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:18 or a sequence having at least about 40% similarity thereto.
53. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:19

or a sequence having at least about 40% similarity thereto.

54. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:20 or a sequence having at least about 40% similarity thereto.

55. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:21 or a sequence having at least about 40% similarity thereto.

56. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein encoded by a nucleotide sequence comprising SEQ ID NO:22 or a sequence having at least about 40% similarity thereto.

57. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein comprising an amino acid sequence set forth in SEQ ID NO:7 or having at least 40% similarity thereto.

58. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein comprising an amino acid sequence set forth in SEQ ID NO:9 or having at least 40% similarity thereto.

59. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein comprising an amino acid sequence set forth in SEQ ID NO:10 or having at least 40% similarity thereto.

60. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein comprising an amino acid sequence set forth in SEQ ID NO:12 or having at least 40% similarity thereto.

61. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein comprising an amino acid sequence set forth in SEQ ID NO:14 or having at least 40% similarity thereto.
62. A method according to claim 38 wherein the immunogenic component comprises a peptide, polypeptide or protein comprising an amino acid sequence set forth in SEQ ID NO:16 or having at least 40% similarity thereto.
63. An isolated nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO:1 or a nucleotide sequence having at least 40% similarity to the nucleotide sequence set forth in SEQ ID NO:1 and which is capable of hybridizing thereto under low stringency conditions and which encodes an immunogenic peptide, polypeptide or protein from *L. intracellularis* or related microorganism.
64. An isolated nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO:3 or a nucleotide sequence having at least 40% similarity to the nucleotide sequence set forth in SEQ ID NO:3 and which is capable of hybridizing thereto under low stringency conditions and which encodes an immunogenic peptide, polypeptide or protein from *L. intracellularis* or related microorganism.
65. An isolated nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO:5 or a nucleotide sequence having at least 40% similarity to the nucleotide sequence set forth in SEQ ID NO:5 and which is capable of hybridizing thereto under low stringency conditions and which encodes an immunogenic peptide, polypeptide or protein from *L. intracellularis* or related microorganism.
66. An isolated nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO:6 or a nucleotide sequence having at least 40% similarity to the nucleotide sequence set forth in SEQ ID NO:6 and which is capable of hybridizing thereto under low stringency conditions and which encodes an immunogenic peptide, polypeptide or protein

from *L. intracellularis* or related microorganism.

67. An isolated nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO:8 or a nucleotide sequence having at least 40% similarity to the nucleotide sequence set forth in SEQ ID NO:8 and which is capable of hybridizing thereto under low stringency conditions and which encodes an immunogenic peptide, polypeptide or protein from *L. intracellularis* or related microorganism.

68. An isolated nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO:11 or a nucleotide sequence having at least 40% similarity to the nucleotide sequence set forth in SEQ ID NO:11 and which is capable of hybridizing thereto under low stringency conditions and which encodes an immunogenic peptide, polypeptide or protein from *L. intracellularis* or related microorganism.

69. An isolated nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO:13 or a nucleotide sequence having at least 40% similarity to the nucleotide sequence set forth in SEQ ID NO:13 and which is capable of hybridizing thereto under low stringency conditions and which encodes an immunogenic peptide, polypeptide or protein from *L. intracellularis* or related microorganism.

70. An isolated nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO:15 or a nucleotide sequence having at least 40% similarity to the nucleotide sequence set forth in SEQ ID NO:15 and which is capable of hybridizing thereto under low stringency conditions and which encodes an immunogenic peptide, polypeptide or protein from *L. intracellularis* or related microorganism.

71. An isolated nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO:17 or a nucleotide sequence having at least 40% similarity to the nucleotide sequence set forth in SEQ ID NO:17 and which is capable of hybridizing thereto under low stringency conditions and which encodes an immunogenic peptide, polypeptide or protein

from *L. intracellularis* or related microorganism.

72. An isolated nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO:18 or a nucleotide sequence having at least 40% similarity to the nucleotide sequence set forth in SEQ ID NO:18 and which is capable of hybridizing thereto under low stringency conditions and which encodes an immunogenic peptide, polypeptide or protein from *L. intracellularis* or related microorganism.

73. An isolated nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO:19 or a nucleotide sequence having at least 40% similarity to the nucleotide sequence set forth in SEQ ID NO:19 and which is capable of hybridizing thereto under low stringency conditions and which encodes an immunogenic peptide, polypeptide or protein from *L. intracellularis* or related microorganism.

74. An isolated nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO:20 or a nucleotide sequence having at least 40% similarity to the nucleotide sequence set forth in SEQ ID NO:20 and which is capable of hybridizing thereto under low stringency conditions and which encodes an immunogenic peptide, polypeptide or protein from *L. intracellularis* or related microorganism.

75. An isolated nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO:21 or a nucleotide sequence having at least 40% similarity to the nucleotide sequence set forth in SEQ ID NO:21 and which is capable of hybridizing thereto under low stringency conditions and which encodes an immunogenic peptide, polypeptide or protein from *L. intracellularis* or related microorganism.

76. An isolated nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO:22 or a nucleotide sequence having at least 40% similarity to the nucleotide sequence set forth in SEQ ID NO:22 and which is capable of hybridizing thereto under low stringency conditions and which encodes an immunogenic peptide, polypeptide or protein

from *L. intracellularis* or related microorganism.

77. A genetic vaccine comprising a DNA sequence having a nucleotide sequence set forth in SEQ ID NO:1 or having at least 40% similarity thereto or is capable of hybridizing to SEQ ID NO:1 under low stringency conditions, said DNA sequence capable of expression in a pig to produce an amount of a peptide, polypeptide or protein effective to induce a protective immune response against *L. intracellularis* or related microorganism.

78. A genetic vaccine comprising a DNA sequence having a nucleotide sequence set forth in SEQ ID NO:3 or having at least 40% similarity thereto or is capable of hybridizing to SEQ ID NO:3 under low stringency conditions, said DNA sequence capable of expression in a pig to produce an amount of a peptide, polypeptide or protein effective to induce a protective immune response against *L. intracellularis* or related microorganism.

79. A genetic vaccine comprising a DNA sequence having a nucleotide sequence set forth in SEQ ID NO:5 or having at least 40% similarity thereto or is capable of hybridizing to SEQ ID NO:5 under low stringency conditions, said DNA sequence capable of expression in a pig to produce an amount of a peptide, polypeptide or protein effective to induce a protective immune response against *L. intracellularis* or related microorganism.

80. A genetic vaccine comprising a DNA sequence having a nucleotide sequence set forth in SEQ ID NO:6 or having at least 40% similarity thereto or is capable of hybridizing to SEQ ID NO:6 under low stringency conditions, said DNA sequence capable of expression in a pig to produce an amount of a peptide, polypeptide or protein effective to induce a protective immune response against *L. intracellularis* or related microorganism.

81. A genetic vaccine comprising a DNA sequence having a nucleotide sequence set forth in SEQ ID NO:8 or having at least 40% similarity thereto or is capable of hybridizing to SEQ ID NO:8 under low stringency conditions, said DNA sequence capable of expression in a pig to produce an amount of a peptide, polypeptide or protein effective to induce a protective

immune response against *L. intracellularis* or related microorganism.

82. A genetic vaccine comprising a DNA sequence having a nucleotide sequence set forth in SEQ ID NO:11 or having at least 40% similarity thereto or is capable of hybridizing to SEQ ID NO:11 under low stringency conditions, said DNA sequence capable of expression in a pig to produce an amount of a peptide, polypeptide or protein effective to induce a protective immune response against *L. intracellularis* or related microorganism.

83. A genetic vaccine comprising a DNA sequence having a nucleotide sequence set forth in SEQ ID NO:13 or having at least 40% similarity thereto or is capable of hybridizing to SEQ ID NO:13 under low stringency conditions, said DNA sequence capable of expression in a pig to produce an amount of a peptide, polypeptide or protein effective to induce a protective immune response against *L. intracellularis* or related microorganism.

84. A genetic vaccine comprising a DNA sequence having a nucleotide sequence set forth in SEQ ID NO:15 or having at least 40% similarity thereto or is capable of hybridizing to SEQ ID NO:15 under low stringency conditions, said DNA sequence capable of expression in a pig to produce an amount of a peptide, polypeptide or protein effective to induce a protective immune response against *L. intracellularis* or related microorganism.

85. A genetic vaccine comprising a DNA sequence having a nucleotide sequence set forth in SEQ ID NO:17 or having at least 40% similarity thereto or is capable of hybridizing to SEQ ID NO:17 under low stringency conditions, said DNA sequence capable of expression in a pig to produce an amount of a peptide, polypeptide or protein effective to induce a protective immune response against *L. intracellularis* or related microorganism.

86. A genetic vaccine comprising a DNA sequence having a nucleotide sequence set forth in SEQ ID NO:18 or having at least 40% similarity thereto or is capable of hybridizing to SEQ ID NO:18 under low stringency conditions, said DNA sequence capable of expression in a pig to produce an amount of a peptide, polypeptide or protein effective to induce a

protective immune response against *L. intracellularis* or related microorganism.

87. A genetic vaccine comprising a DNA sequence having a nucleotide sequence set forth in SEQ ID NO:19 or having at least 40% similarity thereto or is capable of hybridizing to SEQ ID NO:19 under low stringency conditions, said DNA sequence capable of expression in a pig to produce an amount of a peptide, polypeptide or protein effective to induce a protective immune response against *L. intracellularis* or related microorganism.

88. A genetic vaccine comprising a DNA sequence having a nucleotide sequence set forth in SEQ ID NO:20 or having at least 40% similarity thereto or is capable of hybridizing to SEQ ID NO:20 under low stringency conditions, said DNA sequence capable of expression in a pig to produce an amount of a peptide, polypeptide or protein effective to induce a protective immune response against *L. intracellularis* or related microorganism.

89. A genetic vaccine comprising a DNA sequence having a nucleotide sequence set forth in SEQ ID NO:21 or having at least 40% similarity thereto or is capable of hybridizing to SEQ ID NO:21 under low stringency conditions, said DNA sequence capable of expression in a pig to produce an amount of a peptide, polypeptide or protein effective to induce a protective immune response against *L. intracellularis* or related microorganism.

90. A genetic vaccine comprising a DNA sequence having a nucleotide sequence set forth in SEQ ID NO:22 or having at least 40% similarity thereto or is capable of hybridizing to SEQ ID NO:22 under low stringency conditions, said DNA sequence capable of expression in a pig to produce an amount of a peptide, polypeptide or protein effective to induce a protective immune response against *L. intracellularis* or related microorganism.

1/4

395 Y10 Y12 Y14 Y16

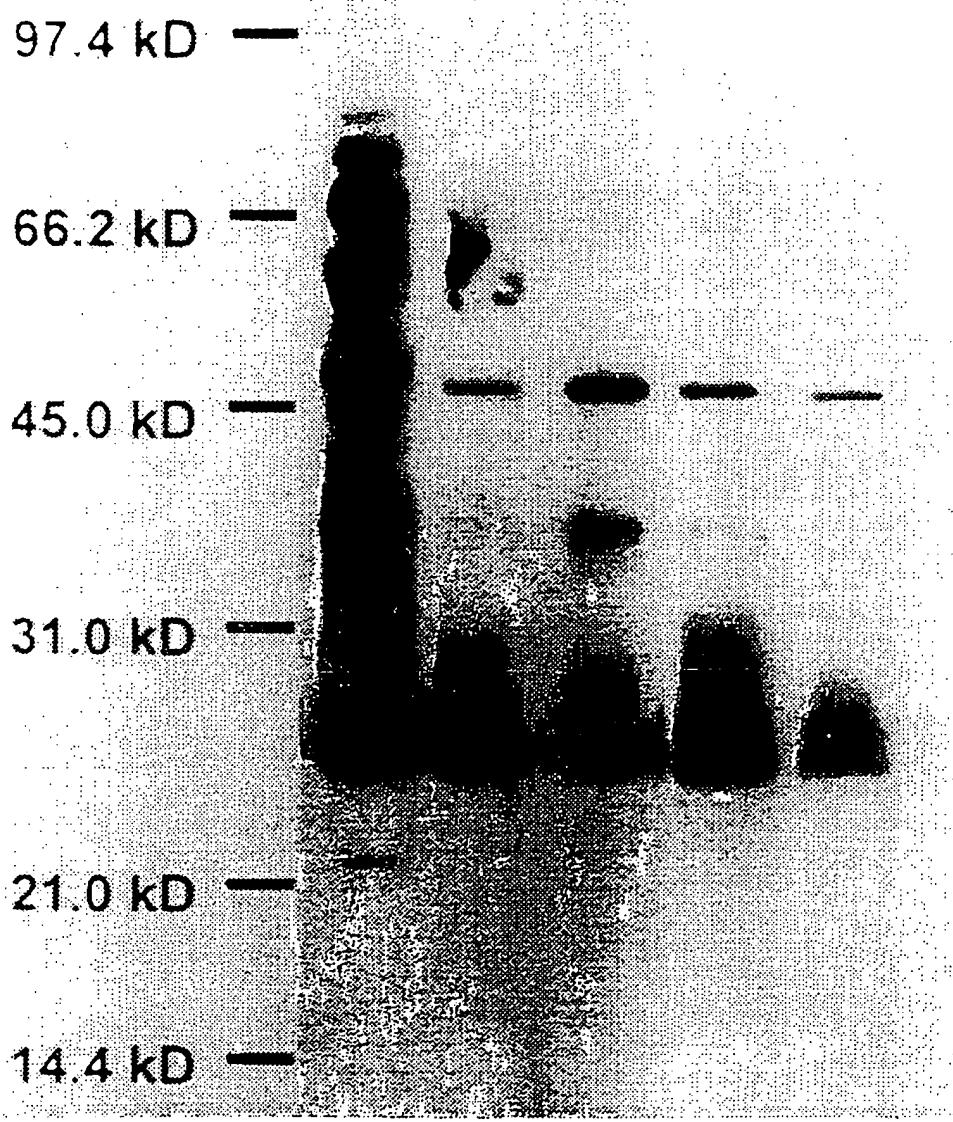


FIG 1

2/4



FIG 2

3/4



FIG 3

4/4



FIG 4

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 96/00767

## A. CLASSIFICATION OF SUBJECT MATTER

Int Cl<sup>o</sup>: C12N 15/31, A61K 39/02, A61K 39/106

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC C12N 15/31, A61K 39/02, A61K 39/106

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
AU:IPC (as above)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Derwent, Chemical Abstracts: lawsonia, intracellularis, ileal, groel, groes, chaperonin  
STN: nucleotide/amino-acid search.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AU, 69290/94, A (Institut Pasteur et al.) 12 December 1994	1, 2, 6, 7, 10, 11, 63, 64, 77, 78
X	Suerbaum et al., "Helicobacter pylori hspA-hspB heat-shock gene cluster: nucleotide sequence, expression putative function and immunogenicity", Molecular Microbiology, Vol. 14, No. 5, 1994, pages 959-974, see entire document.	1, 2, 6, 7, 10, 11, 63, 64, 77, 78



Further documents are listed in the continuation of Box C



See patent family annex

• Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier document but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search  
13 February 1997

Date of mailing of the international search report

26 FEB 1997

Name and mailing address of the ISA/AU  
AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION  
PO BOX 200  
WODEN ACT 2606  
AUSTRALIA Facsimile No.: (06) 285 3929

Authorized officer

R.L. POOLEY

Telephone No.: (06) 283 2242

## INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/AU 96/00767

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Kansau et al., "Heat shock proteins of <i>Helicobacter pylori</i> ". Aliment. Pharmacol. Ther., Vol. 10, Suppl. 1, 1996, pages 51-6, see entire document.	1, 2, 6, 7, 10, 11, 63, 64, 77, 78
X	Wu et al., "Heat Shock- and Alkaline pH-Induced Proteins of <i>Campylobacter jejuni</i> : Characterization and Immunological Properties", Infection and Immunity, Vol. 62, No. 10, 1994, pages 4256-4260, see entire document.	1, 2, 6, 7, 10, 11, 63, 64, 77, 78
X	Dunn et al., "Identification and Purification of a cpn 60 Heat shock Protein Homolog from <i>Helicobacter pylori</i> ", Infection and Immunity, Vol. 60, No. 5, 1992, pages 1946-1951, see entire document.	63, 77
X	Evans et al., "Urease-Associated Heat Shock Protein of <i>Helicobacter pylori</i> ", Infection and Immunity, Vol. 60, No 5, 1992, pages 2125-2127, see entire document.	63, 77
X	Takata et al., "The Purification of a GroEL-Like Stress Protein from Aerobically Adapted <i>Campylobacter jejuni</i> ". Microbiol. Immunol., Vol. 39, No. 9, pages 639-645, see entire document.	63, 77
X	Bukanov et al., "Ordered cosmid library and high-resolution physical-genetic map of <i>Helicobacter pylori</i> strain NCTC11638", Molecular Microbiology, Vol. 11, No. 3, 1994, pages 509-523.	63, 77

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

International Application No.  
PCT/AU 96/00767

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
AU, A	69290/94	WO,	94/26901	EP,	703981
		JP,	8510120		CA, 2144307

END OF ANNEX